NEW ZEALAND
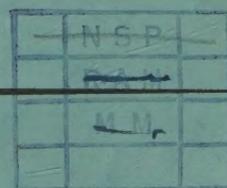# JOURNAL
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A NEW ZEALAND PHYTOCHEMICAL SURVEY –
PART I. THE GYMNOSPERMS

By B. F. Cain, S. Scannell, British Empire Cancer Campaign Society Laboratory, Cornwall Hospital, and R. C. Cambie, Department of Chemistry, University of Auckland.

Summary

Gymnosperms endemic to New Zealand have been examined for the presence of alkaloids, leucoanthocyanins, saponins, and triterpenes or steroids. They all contain leucoanthocyanins, the derived anthocyanidins of which have been identified by paper chromatography. A single species, *Agathis australis,* contains an alkaloid in the heartwood.

INTRODUCTION

During the course of a survey of the New Zealand native flora for possible tumour inhibitory principles, extracts have been examined for the presence of alkaloids, leucoanthocyanins, saponins, and triterpenes or steroids. In the latter work numerous fresh plants not extracted in bulk, and also herbarium specimens from the Auckland Institute and Museum, have been examined. The results of testing the New Zealand gymnosperms are now reported.

In this and subsequent papers of this series the methods of testing for alkaloids, saponins, and triterpenes follow the more comprehensive surveys carried out on the Australian flora (Webb, 1949, 1952; Simes, Tracey, Webb and Dunstan, 1959) and that of Papua - New Guinea (Webb, 1955). A similar survey for the presence of alkaloids in the Hawaiian flora has been initiated by Swanholm and co-workers (1959, 1960). The investiga-

tion of leucoanthocyanins in the native flora is based on the study of a large number of dicotyledonous plants by Bate-Smith and Metcalfe (1957). All species of gymnosperms considered in this communication are endemic to New Zealand.

Most of the samples tested have been collected from fresh plants in the Auckland or central North Island areas and, where necessary, leaf specimens of samples tested have been lodged in the herbarium of the Auckland Institute and Museum to aid future identification. Herbarium samples of some species have also been tested and found to give results identical with those obtained from the fresh plants.

Methods

Extraction of Plant Material

A dried, finely ground sample was extracted (Soxhlet) with methanol, the extract concentrated, and final traces of solvent removed, *in vacuo*. A portion of the residue, which was usually a red or brown friable solid, was used directly for testing. In the case of herbarium samples and where plant material was available in limited supply a finely chopped portion of the plant (1-2 g) was tested without prior extraction with methanol.

Alkaloids

A portion of the residue or plant material was digested in a test-tube (3 in. $\times$ $\frac{1}{2}$ in.) with 2N-aqueous hydrochloric acid saturated with sodium chloride, for periods of 15–30 min., the mixture gravity filtered through fine paper and the filtrate tested with the alkaloid reagents. Information obtained by digesting plant material with Prollius' fluid gave no further information than obtained by acid digestion and was not generally used (cf. Swanholm *et al.*, 1960).

A number of the usual alkaloid precipitating reagents (Cromwell, 1955) were used in initial testing but restriction to the use of Dragendorff's and Mayer's reagents has normally been found to give reliable results and the use of other precipitants was therefore discontinued. Also, reagents such as Wagner's and Bouchardat's suffered from the disadvantage of giving precipitates with non-alkaloidal material while Hager's reagent showed low sensitivity.

Leucoanthocyanins

A portion of the residue or plant material was digested for 15–30 min. with 2N-hydrochloric acid in propan-1-ol (cf. Bate-Smith and Metcalfe, 1957). The slow development of a strong red or violet coloration was recognised as a positive test and the concentrated anthocyanidins could readily be identified by comparative circular paper chromatography with authentic samples, using Forestal's solvent system.

Saponins

A portion of the residue or plant material was digested with boiling water for 15–30 min. in a small test-tube (3 in. × ½ in.) and then shaken vigorously for 3–5 min. In all cases recorded as giving a positive froth test, the froth had a characteristic honeycomb appearance and was stable for at least 30 min. A number of plants, in subsequent parts of this series, gave small froths stable for a few minutes and although they may contain small amounts of saponin, the tests have been recorded as negative.

Triterpenes

Liebermann-Burchard tests carried out in solution were used as a guide to the presence of triterpenes and steroids. A portion of the residue or plant material was digested with a reagent prepared by mixing acetic anhydride-concentrated sulphuric acid-chloroform (10 : 1 : 25 by volume), for 1–2 min. The test was regarded as positive when strong red, pink, purple, violet or blue colours were produced. No attempt has been made to distinguish between triterpenes and steroids in the present communication (cf. Simes *et al.* 1959).

KEY TO ABBREVIATIONS IN TABLE 1

The results of the tests are given in the following order and abbreviated form:

Plant Name

The name used in Cheeseman (1925) or subsequent revisions is given.

Maori Name

The Maori name is that given by Cheeseman (1925) or by Anderson (1926). Where the Maori name is unknown the popular name is recorded.

Plant Part

B = bark, C = cone, H = whole plant, L = leaves, R = root, RB = root bark, RW = root wood, T = twigs, W = wood.

Locality

General area of collection of the fresh plant. Auckland refers to samples collected from the grounds of the University of Auckland. Herbarium refers to a sample from the herbarium of the Auckland Institute and Museum.

Month Collected

Month of actual collection in the field. Testing was carried out in most cases within a few days of collection.

Herbarium Number

Auckland Institute and Museum herbarium number of a leaf specimen actually tested and lodged in the herbarium or the number of a specimen taken from the herbarium for testing.

Alkaloid Test

M = Mayer's reagent, D = Dragendorff's reagent. Precipitates are classified as positive (+) or negative (−).

Leucoanthocyanins Test

LA = Leucoanthocyanin. Tests are classified as positive or negative.

Saponin Test

Sap. = Saponin. Tests are classified as positive or negative.

Liebermann-Burchard Test

LB = Liebermann-Burchard. Tests are classified as positive or negative.

Results and Discussion

The results of testing are recorded in Table 1. Full discussions of the methods used, the limitations of the tests and of the use of herbarium samples have been made by Webb (1949, 1952), Simes *et al.* (1959) and by Bate-Smith and Metcalfe (1957).

Alkaloids are not commonly found in species of the gymnosperms, notable exceptions being ephedrine from *Ephedra* species and taxine from *Taxus baccata* (Henry 1949). No species of the New Zealand Cupressaceae or Podocarpaceae were found to contain alkaloids but the heartwood of the sole representative of the Pinaceae, *Agathis australis,* was found to give positive tests. The presence of an alkaloid has been subsequently confirmed when bulk samples of the heartwood were extracted and a water-insoluble alkaloidal fraction isolated in low yield. Attempts to isolate a pure compound have been unsuccessful.

All the species gave positive tests for leucoanthocyanins, the barks of the plants being the richest source of these compounds. The isolation of the principal leucoanthocyanin from the bark of *Dacrydium cupressinum* has already been reported (Cambie and Cain, 1959) and has been shown to be a stereoisomer of leucocyanidin which is apparently not identical with any isomer previously isolated. That from the bark of *Dacrydium biforme* has also been isolated by one of the authors (R.C.C.), but has not been obtained pure. It differs from that of *D. cupressinum,* giving delphinidin on hydrolysis.

TABLE 1

Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap.	LB
PINACEAE										
Agathis australis Salisb.	kauri	L	Waitakeres	Dec.		−	−	+	−	+
		W	"	March		+	+	+	−	+
		RW	"	Dec.		−	−	+	−	+
		B	"	March		−	−	+	−	−
		RB	"	Dec.		−	−	+	−	−
		C				−	+	+	−	+
CUPRESSACEAE										
Libocedrus bidwillii Hook. f.	pahautea	L, T	Nat. Park	Jan.		−	−	+	−	−
		W	"	?		−	−	+	−	−
		B	"	Feb.		−	−	+	−	−
Libocedrus plumosa (Don) Sarg. (*L. doniana* Endl.)	kawaka	L	Waitakeres	March	46476	−	−	+	−	−
		W	"	"		−	−	+	−	−
		RW	"	"		−	−	+	−	−
		B	"	"		−	−	+	−	−
		RB	"	"		−	−	+	−	−
PODOCARPACEAE										
Dacrydium bidwillii Hook. f.	mountain pine	L	Nat. Park	Nov.	50137	−	−	+	−	−
		W	"	"		−	−	+	−	+
		RW	"	"		−	−	+	−	−
		B	"	"		−	−	+	−	−
		RB	"	"		−	−	+	−	−
Dacrydium biforme Pilger	yellow pine	L	Nat Park	June		−	−	+	−	+
		W	"	?		−	−	+	−	+
		B	Auckland.	Sept.		−	−	+	−	+

TABLE 1—continued

Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test		LA	Sap.	LB
						M	D			
Dacrydium colensoi Hook.	silver pine	L	Nat. Park	Oct.	50060 & 50099	−	−	+	−	+
		W	″	″		−	−	++	−	+
		B	″	″		−	−	++	−	−
Dacrydium cupressinum Soland.	rimu	L	Waitakeres	March		−	−	+	−	+
		W	″	″		−	−	+	−	+
		RW	″	″		−	−	+	−	+
		B	″	″		−	−	+	−	+
		RB	″	″		−	−	+	−	+
Dacrydium intermedium T. Kirk	yellow silver pine	L, T	Herbarium	—		−	−	+	−	−
Dacrydium kirkii F. Muell.	monoao	L	Auckland	Sept.		−	−	+	−	−
		W, B	″	″		−	−	+	−	−
Dacrydium laxifolium Hook. f.	pigmy pine	H	Nat Park	Sept., Nov.	50109	−	−	+	−	+
Podocarpus acutifolius T. Kirk	sharp leaved totara	L	Auckland	Sept.		−	−	+	−	−
		T	Herbarium	″		−	−	+	−	−
		W, B	″	—	70129	−	−	+	−	−
Podocarpus dacrydioides A. Rich.	kahikatea	L	Waitakeres	March		−	−	+	−	−
		W	″	″		−	−	+	−	−
		B	″	″		−	−	+	−	−
Podocarpus ferrugineus D. Don	miro	L	Waitakeres	March		−	−	+	−	+
		W	″	″		−	−	+	−	−
		B	″	″		−	−	+	−	−
		RB	″	″		−	−	+	−	−

Species	Common name	Part	Locality	Month	Number
Podocarpus nivalis Hook.	tauhinu	L, T	Australia	April	46496
Podocarpus nivalis Hook. var. *erectus* cockayne	(alpine totara)	L	Nat. Park	Oct.	50055
		W	"	"	
		B	"	"	
		R	"	"	
Podocarpus spicatus R. Br.	matai	L, T	L. Rotaira	Dec.	50100
		W	"	"	
		B	"	"	
Podocarpus totara D. Don	totara	L	Waitakeres	Feb.	46453 & 46475
		W	"	March	
		RW	"	March, Feb.	
		B	"	"	
		RB			
Podocarpus hallii T. Kirk (*P. totara* var. *hallii* Pilger)	fuchsia-barked totara	L	Auckland	Sept.	
		W, B	"	"	
Phyllocladus alpinus Hook. f.	alpine toatoa	L	Nat. Park	Oct.	50054
		W	"	Nov.	
		B	"	Oct.	
Phyllocladus glaucus Carr.	toatoa	L	Waitakeres	March	
		W	"	"	
		B	"	"	
		RB	"	"	
Phyllocladus trichomanoides D. Don	tanekaha	L	Waitakeres	March	46451
		W	"	"	
		RW	"	"	
		B	"	"	
		RB	"	"	

The derived anthocyanidins from a number of samples were identified by circular paper chromatography and the relative strengths of leucoanthocyanins estimated visually on a weak (x) to strong (xxx) basis. Results are reported in Table 2. Cyanidin had R_f 0·54, delphinidin R_f 0·41, and pelargonidin R_f 0·69, using Forestal's solvent system.

Table 2

Name of Plant	Plant Part	L-D	L-Cy	L-P
Agathis australis	B	x	x	
	W		x	
Libocedrus bidwillii	B		xxx	
	W		x	
Libocedrus plumosa	B		xxx	
	W		x	
Dacrydium bidwillii	B		xx	
	W		x	
Dacrydium biforme	L, T	x		
	B	xx		
Dacrydium colensoi	B		xx	
	W		x	
Dacrydium cupressinum	B		xx	
	W		x	
Dacrydium intermedium	L, T		x	
Dacrydium kirkii	L, T	xx		
Dacrydium laxifolium	L, T	x		
Podocarpus acutifolius	L, T		x	
Podocarpus dacrydioides	B	x	xxx	x
	W		x	
Podocarpus ferrugineus	B		xx	
	W		x	
Podocarpus nivalis	B	xx	x	
Podocarpus hallii	L, T		x	
Podocarpus spicatus	B		x	
Podocarpus totara	B	x	xx	
	W	x	x	
Phyllocladus alpinus	B		x	
	W		x	
Phyllocladus glaucus	B		x	
	W		x	
Phyllocladus trichomanoides	B		x	
	W		xx	

No sample of the species examined gave a positive froth test for saponins but a number gave colorations in the Liebermann-Burchard tests. β-Sitosterol, which gives a positive Liebermann-Burchard test, has been isolated in some cases viz, from the wood (Brandt and Thomas, 1951) and the bark (Cambie and Cain, 1960) of *Dacrydium cupressinum*, and from *Dacrydium biforme* bark and *Podocarpus totara* heartwood by one of the authors (R.C.C.) and L. N. Mander (pers. comm.) and the weak positive tests given by the barks and heartwoods of other species in this series may be due to the presence of this compound. Strong yellow-brown colorations were given by the leaves of all species known to contain diterpenes and have been recorded here as positive tests. The colour which is also given by pure samples of the hydrocarbons, phyllocladene, isophyllocladene, kaurene, isokaurene, rimuene, and cupressene is undoubtedly due to the presence of these compounds and probably not to the presence of triterpene or steroidal material.

ACKNOWLEDGMENTS

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SOPHORA ALKALOIDS. PART 7

THE ALKALOIDS OF S. TOMENTOSA

By R. C. CAMBIE, Department of Chemistry, University of Auckland.

Summary

The seeds and leaves of *Sophora tomentosa* have been shown to contain matrine, cytisine, a little methylcytisine, and in trace amount, an unidentified base.

INTRODUCTION

Plugge (1894, 1895) and Plugge and Rauwerda (1896) have shown that the species *Sophora tomentosa* contains the alkaloid cytisine. A re-examination of the seeds and leaves of this species from a tree growing in Australia by methods similar to those described in earlier parts of this series (Briggs and co-workers, 1937, 1938, 1942, 1948, 1960) has now shown the presence of four alkaloids. α-Matrine and cytisine constitute the principal bases and occur in approximately equal amount in both the seeds and the leaves while methylcytisine is present in lesser amount. The fourth base (D) which is present only in trace amount has not been identified. Comparative paper chromatography showed that it was not identical with anagyrine or sophochrysine, alkaloids which have been found in New Zealand *Sophora* species. The identified bases or their derivatives have been isolated from the seeds and identified in the leaves by circular chromatography. Cytisine could be readily separated from other alkaloids in the seeds by partition column chromatography. The melting point of α matrine perchlorate, prepared by an improved method has been raised considerably above that previously recorded.

EXPERIMENTAL

Infrared spectra were measured as KBr discs. Circular paper chromatography of alkaloid fractions and authentic bases was carried out with the organic phase of the following solvent systems: (A) butan–1–ol—water—36% HCl (50:17:7·5), (B) butan–2–ol—water—glacial acetic acid (50:17:2), (C) butan–1–ol—water (10:3), and using Dragendorff's reagent as a spray. R_f values for solvent C followed the order given by White (1957) but in this and other solvents R_f values were found to be markedly dependent upon the concentration of the initial spots, those of least concentration (just detectable) moving up to 20% faster than spots of maximum concentration (not quite tailing). All comparisons were there-fore made against a range of authentic samples and R_f values are not reported.

Isolation of the Alkaloids

The crushed seeds of *Sophora tomentosa* (600 g), were extracted (Soxhlet) with methanol and the residue, obtained on removal of solvent, extracted with 2N-aqueous hydrochloric acid. The filtered solution was basified with concentrated ammonium hydroxide and continuously extracted with chloroform until the aqueous phase gave only a weak alkaloid test with Mayer's reagent. The chloroform solution was dried (anhyd. $CaCl_2$) and the solvent removed to yield a crude alkaloid fraction as a brown viscous oil (19·2 g; 3% yield).

Extraction of the leaves (290 g) in the same manner gave crude bases as a brown viscous oil (7.3 g; 2·5% yield). Paper chromatographic investigation showed the presence of α-matrine, cytisine, methylcytisine and base D in both the leaf and seed fractions. That from the seeds was distilled and a single fraction collected, b.p. 163–185/0·01 mm. The mixed bases which solidified on standing or on contact with light petroleum were exhaustively extracted (Soxhlet) with light petroleum (b.p. < 40°) which dissolved mainly α-matrine and some methylcytisine (paper chromatography). The insoluble residue consisted mainly of cytisine, a little methylcytisine and α-matrine, and a trace of base D.

α-Matrine

Repeated fractional crystallisation of the soluble fraction from light petroleum (b.p. < 50°) gave α-matrine (402 mg) as colourless needles, m.p. and mixed m.p. 76°–77° (identical infrared spectrum). Matrine aurichloride, needles from hot water, had m.p. and mixed m.p. 196°–197° (decomp.). The perchlorate was prepared by adding 60% aqueous perchloric acid to matrine and heating at 100° for a few minutes. The crystalline product (quantitative yield) which deposited on cooling, after recrystallisation from chloroform-60% perchloric acid as large prisms, had m.p. 267°–268° with sintering below 200° and slow decomposition from 251°. Briggs and Russell (1942) record m.p. 214·5°–216°.

Cytisine

The insoluble residue was chromatographed on a column of cellulose powder (Whatman standard grade) previously moistened with phosphate buffer pH 7·4. Fractions, eluted with solvent C were collected automatically at intervals of ½ min, and examined by paper chromatography. Fractions 16–21 contained matrine (trace), methylcytisine, and base D (trace), fractions 22–23, methylcytisine and cytisine, and fractions 24–42, cytisine.

Cytisine (326 mg) crystallised directly from fractions 24–42 on removal of solvent, *in vacuo,* and, after further purification from butan–1–ol, had m.p. and mixed m.p. 152°–153° (identical infrared spectrum). Further cytisine was recovered from the mother liquors as the benzene sulphonate (230 mg) which crystallised from ethanol as plates, m.p. and mixed m.p. 261°–262°. Cytisine perchlorate, needles from ethanol, had m.p. 308° with slow decomposition from 295°. Briggs and Russell (1942) record m.p. 296° (decomp.).

geneous and exogenous fat. The former is of lower iodine value and comprises a larger proportion of the fast growing tissues than the exogenous fat derived from the relatively high iodine value dietary fat. Subsequent studies on sheep (Callow, 1958) and on cattle (Callow and Searle, 1956) provided no clear-cut relationship between growth rate and iodine value, but suggested other interesting correlations such as between order of development of the tissue and iodine value. However, such correlations were less definite than those found between iodine value and growth rate of the pig, and were, in addition, overshadowed by the fact that individuality was the major variable.

The difficulty of obtaining a complete explanation of the changes in composition of fats with site of deposition is further indicated by relatively slight differences in the iodine values between the external and internal fats of horses as compared with those of pigs, sheep, and oxen (*cf.* Dahl, 1958).

Although routine tests are normally conducted at the Freezing Works to check the quality of tallows and neatsfoot oils, little systematic work has been published on the characteristics of fats from New Zealand sheep and cattle. (Wright, 1921a) has given the range of specific gravities, melting points, titres, saponification values and free fatty acid contents of tallows and neatsfoot oils generally as well as the melting points, titres, and free fatty acid contents of typical edible mutton and beef tallows (Wright, 1921b). In addition, Wright and Thompson (1927) found that the average titres of mutton tallows varied with the geographical latitude. Sheep living at 40° South produced tallows with an average titre of 47·9°C, whereas those living at 46° South yielded tallows with an average titre of 44·7°C. Barnicoat and Shorland (1952) reported the iodine values of the fats from the various joints of the export grades of North Island lamb and mutton. Kidney fats were found to possess iodine values some 5–9 units lower than those from the joints. The iodine values of the total fat also varied according to the type of carcase as follows: lamb, 48·5; wethers, 46·1; ewes, 44·2.

Detailed knowledge of the properties of the fats from New Zealand mutton and beef animals is thus lacking, and in particular there is practically no information about the properties of the subcutaneous fats. It could be expected that the subcutaneous fats are intermediate in their properties between the soft hoof oils and the hard kidney fats. However, as might be deduced from the facts mentioned above, only experimental work can clarify the position.

The potential economic value of New Zealand low melting fats is considerable. The great abundance of high melting animal fats such as beef and mutton tallows produced in this country contrasts with an almost total absence of liquid fats of medium iodine value (except the neatsfoot oil, and small amounts of horse fat) which presents problems in certain fat using industries. On the other hand fats with a high melting point are at a premium in overseas countries, including Great Britain, the main consumer of New Zealand tallows, and the inclusion of low melting subcutaneous and similar fats in beef and mutton tallows tends to detract from the export value of these products.

The present work was undertaken to determine the usual physical and chemical characteristics and the fatty acid composition of subcutaneous fats from sheep and oxen and to compare them with those established here and overseas for perinephric fat. It is hoped that the data thus obtained may pave the way towards recovery and better utilisation of subcutaneous fats which at present are rendered together with other fatty tissues in New Zealand meat works. Apart from the immediate practical value of the work, it is hoped that the data now provided will contribute towards understanding the nature of the variations in fatty acid composition in the different parts of animals.

<div align="center">EXPERIMENTAL</div>

Subcutaneous Fats from Oxen

Samples of fatty tissues (500–1,000 g) were collected by cutting thin slices of fat from the skins of freshly killed, (a) 8 "boner" cows, (b) 3 oxen and 3 cows, and (c) from the tails of 5 "boner" cows. The samples were washed thoroughly with ice-cold water and put through a mincer. The mince was dehydrated and partly extracted with boiling acetone and the residual tissue was extracted exhaustively with a 1 : 10 mixture of methanol and light petroleum (b.p. 40°–50°) in a Soxhlet. After the evaporation of the solvents from both extractions the fat was taken up in light petroleum which was subsequently evaporated first under atmospheric pressure and finally *in vacuo*. For comparison, a minced sample of subcutaneous fat from "boner" cows was steam rendered for 2 hours at a pressure of 45 lb per sq. in. in a laboratory autoclave. The rendered fat was drained from the residue and filtered.

In addition, 2 samples of steam rendered subcutaneous fat were kindly supplied by Messrs A. E. Preston Ltd., Wellington, for the estimation of stability against oxidation.

Subcutaneous Fats from Sheep

A sample of commercially rendered subcutaneous fat was acquired through the courtesy of Messrs Gear Meat Co. Ltd., Wellington. This fat was obtained from sheep pelts treated with depilatory agents and was purified by boiling with diluted sulphuric acid to remove lime and other chemicals. As analysis showed that this fat was probably contaminated (woolgrease, skin fat, etc.) samples of unadulterated subcutaneous fat were subsequently obtained from four skins of, (a) 3 male and 3 female lambs, and (b) 6 ewes and 6 wethers. These samples were solvent extracted as described in the preceding section.

The Characteristics of Fats and of Fatty Acids Obtained Therefrom

The acid values, saponification equivalents, iodine values, unsaponifiable matter, and refractive indices of these fats were determined by standard methods. For the determination of the melting (slip) points the open

capillary method (British Standard methods of Analysis of Oils and Fats) was used. The induction periods of the steam rendered subcutaneous "boner" cow fat and of the two commercial samples were determined by the accelerated stability test (King, et al., 1933). After the saponification of 20 g samples of the fats specified before and the removal of the un-saponifiable matter as described by Hilditch (1956) the free fatty acids were recovered and their melting points (capillary method), titres, saponification equivalents, and iodine values were determined.

Fatty Acid Composition of Subcutaneous Fats

After their conversion into methyl esters, as described by Hilditch (1956), the composition of fatty acids was established by gas-liquid chromatography using a Pye Argon Chromatograph (Cambridge). The chromatographic columns had a length of 1,200 mm and an internal diameter of 4 mm and packings with two different liquid phases were employed. One set of columns was packed with 45–85 mesh Celite 545 mixed with 20% (w/w) Apiezon M (James and Martin, 1956) and operated at 200°. In the second set, Celite was impregnated with 20% (w/w) polyethylene glycol succinate (Lipsky and Landowne, 1958) and the working temperature was 150°. The first set of columns made possible the separation of saturated from unsaturated methyl esters, whereas the second packing was used to resolve mono- di- and tri- unsaturated esters of the C_{18} series. No satisfactory separation, however, was achieved, and the amounts of di- and poly-unsaturated acids were therefore determined by the alkali isomerisation of Brice et al (1952).

Results and Discussion

The acid values and other characteristics of ox and sheep subcutaneous fats and of their fatty acids are given in Table 1. For comparison, a few data for perinephric fats determined on New Zealand commercial tallows and taken from the literature are given in the same table. The values agree with those obtained earlier by Wright (1921a). Whereas acid values for New Zealand tallows represent, on the whole, a variable characteristic of fats, the low acid values of both ox and sheep subcutaneous fats prepared in the laboratory indicate that, given suitable treatment, these fats can be obtained in an edible quality. In conformity with the findings of overseas workers (cf. Dahl, 1958), the iodine values of the subcutaneous fats and fatty acids are appreciably higher than those of perinephric fats, whereas the opposite is true for their melting points and titres.

Differences in iodine value between subcutaneous and perinephric fats are more pronounced in oxen than in sheep, indicating species differences. In horses (Shorland, Bruce, and Jessop, 1952) and in birds (Hilditch. 1956), these differences are almost non-existent. These divergences render the formulation of a general theory to account for variations in iodine value in terms of growth rate or temperature gradient difficult.

The fatty acid compositions of the various fats are summarised in Table 2. As already mentioned the data are based mainly on gas-liquid chromatographic analysis, but also include the results of the ester fractionation

TABLE 1—Characteristics of Subcutaneous Fats and Fatty Acids from Oxen and Sheep Compared With Perinephric Fats

Specimen	Sample	Date Collected	Nos. and Sex of Specimens	Ref. Index $n^{60°c}$	Characteristics of Fats					Characteristics of Fatty Acids			
					M. pt. (°c)	Sap. eq.	Iod. Value	Unsap. Matter	Free Fatty Acid as Oleic (%)	M. pt. (°c)	Titre (°c)	Neutr. Eq.	Iodine Value
"Boner cow"	N52 subcut. steam rend.	27.V.59	8 female	1.4529	35.8	282.8	56.4	0.35	0.27	36–37	34.7	267.2	59.5
"Boner cow"	N52 subcut. solv. extr.	27.V.59	8 female	1.4529	37.0	283.1	54.7	0.50	0.27	39–40	36.6	268.6	57.9
"Boner cow"	N53 Tail fat	27.V.59	5 female	1.4528	35.0	281.5	54.1	0.50	0.32	37–38	34.7	268.3	60.2
Ox	N54 subcut.	28.V.59	{3 male 3 female}	1.4533	28.8	284.0	61.2	0.46	0.35	36.5–37.5	33.1	271.5	64.2
Sheep	N109 subcut.	23.XII.59	{6 male 6 female}	1.4524	41.5	286.2	52.0	0.43	0.19	41.5–42.5	39.6	275.9	54.1
Lamb	N108 subcut.	23.XII.59	{3 male 3 female}	1.4524	39.6	287.6	50.2	0.46	0.31	41–42	38.0	270.1	51.4
Sheep	N51 subcut. commcl.	27.V.59		1.4524	42.1	284.7	51.2	0.80	2.53	42.5–43.5	40.8	267.2	54.9
Ox	Perineph. commercial Wright (1921a)			1.4509	46.8 / 41–48	283.7 / 283–286.5	39.7	0.58	0.50	45.5–46.5	43.8 / 43–45	271.2	40.7
Sheep	Perineph. commercial Wright (1921a)			1.4517	46.3 / 42–49	286.5 / 287–292	44.3	0.54	0.45	47.0–48.0	45.0 / 43–46	273.2	46.5

TABLE 2—Fatty Acid Composition of Subcutaneous Fats from Oxen and Sheep Compared with Perinephric Fats

COMPONENT FATTY ACIDS

Specimen	Sample	Method of Analysis	Saturated									Unsaturated				
			C_{10}	C_{12}	C_{14}	C_{15} (br)	n-C_{15}	C_{16}	C_{17}	C_{18}	C_{20}	C_{14}	C_{16}	C_{17}*	C_{18}	C_{20}
Beef subcutaneous	N52 steam rendered	Gas liquid			2.5† / 3.0‡			22.4 / 23.8	0.6 / 0.6	11.5 / 10.9		1.6 / 1.9	6.7 / 7.3	1.8 / 1.8	52.9 / 50.7	
subcutaneous	N52 solvent extracted	Gas liquid			2.3 / 2.8			22.4 / 23.8	0.8 / 0.8	13.5 / 12.9		1.1 / 1.3	5.9 / 6.3	1.6 / 1.6	52.4 / 50.5	
subcutaneous	N53	Gas liquid			2.3 / 2.8			19.6 / 20.8	0.9 / 0.9	14.9 / 14.3		1.4 / 1.7	6.3 / 6.8	2.2 / 2.2	52.4 / 50.5	
subcutaneous	N54	Gas liquid			2.1 / 2.5			24.9 / 26.3	— / —	8.6 / 8.2		1.3 / 1.6	8.7 / 9.2	2.8 / 2.8	51.6 / 49.4	
subcutaneous	N54	Ester fractionation			2.6 / 3.1			24.6 / 25.9	— / —	7.2 / 7.1		2.1 / 2.5	7.7 / 8.1	— / —	55.3 / 52.8	0.6 / 0.5
Beef perinephric(§)		Ester fractionation		0.1 / 0.1	3.3 / 3.9	0.4 / 0.5	0.4 / 0.5	27.0 / 28.7	— / —	31.7 / 30.4	0.7 / 0.6	0.4 / 0.5	1.7 / 1.8	— / —	34.2 / 33.2	0.9 / 0.8
Sheep subcutaneous	N109	Gas liquid			1.7 / 2.1	0.4 / 0.5	0.4 / 0.4	20.3 / 21.7	2.3 / 2.3	21.1 / 20.4			1.9 / 2.1	2.2 / 2.2	49.7 / 48.2	
Lamb subcutaneous	N108	Gas liquid	0.5 / 0.8	0.8 / 1.1	7.4 / 8.7	0.2 / 0.2	0.4 / 0.4	23.2 / 24.3	0.8 / 0.8	15.3 / 14.5			1.7 / 1.8	1.1 / 1.1	48.6 / 46.3	
Sheep perinephric (Hilditch and Pedelty, 1941)		Ester fractionation			2.9 / 3.5	—		24.0 / 25.6	—	24.9 / 23.9		0.7 / 0.8	2.4 / 2.6	—	44.4 / 43.0	0.7 / 0.6

(br) Branched chain acid. *Contains some saturated branched chain acids. †Weight %. ‡Mole %. §Data from earlier investigations by R. P. Hansen and F. B. Shorland.

method. The results from both methods show a satisfactory degree of agreement, the only appreciable difference being due to the presence of C_{17} acids which is normally not revealed by the ester fractionation method. No di- or poly-unsaturated acids have been identified by gas-liquid chromatography, but the presence of minor amounts of these acids is indicated by the results of the spectrophotometric examination as shown in Table 3. The correlation between gas-liquid chromatographic and spectrophotometric results for di- and poly-unsaturated acids in this laboratory has not been on the whole satisfactory, contrary to the recent findings of Insull and Ahrens (1959) relating to fatty acids of human milk. Significant discrepancies between the results achieved by the two methods have been also reported by Craig and Murty (1959) for some vegetable oils. Craig and Murty regard the gas-liquid chromatographic results as reliable, but it seems that further work is required to resolve the above mentioned differences.

TABLE 3—The Contents of Di- and Tri-unsaturated Acids in Subcutaneous Fats From Oxen and Sheep

| Specimen | Sample | Dienoic and Trienoic Acids % | | | |
| | | Unconjugated | | Conjugated | |
		Diene	Triene	Diene	Triene
Ox	N52 steam rend.	0.4	1.0	1.1	0.04
,,	N52 solv. extr.	1.8	1.1	1.1	0.05
,,	N53	0.6	0.7	1.0	0.04
,,	N54	0.4	1.0	1.3	0.04
Sheep	N109	0.2	1.1	2.4	0.07
Lamb	N108	1.1	1.4	2.5	0.07
Sheep	N51 (commercial)	0.6	1.7	2.6	0.07

Steam rendering and solvent extraction yielded fats differing in their melting points and fatty acid composition (cf. Tables 1 and 2). The steam-rendered fat had a lower melting point and contained a higher percentage of unsaturated fatty-acid components. It appears, however, that although the method of fat recovery had an effect on the characteristics of the product obtained, these differences are of no great commercial importance.

The low melting points of subcutaneous beef and sheep fats are largely due to higher proportions of oleic acids and lower proportions of saturated acids in these fats than in perinephric fats, although the glyceride composition and configuration may also play a part. In addition, there is a substantial increase in palmitoleic acid in subcutaneous beef fat. However, the amounts of palmitoleic acid found in New Zealand beef subcutaneous fats, although much higher than in perinephric fats (7–9% against 2–4%) are considerably lower than those reported by Dahl (1957a, 1957b), i.e., 13% and more. This might be due to New Zealand climatic, and possibly also, feeding conditions. The content of palmitoleic acid in sheep and lamb subcutaneous fats is similar to that of the perinephric fat.

Besides the appreciable difference in its content of palmitoleic acid, the beef subcutaneous fat differs from perinephric fat in the high ratio of palmitic to stearic acid, i.e., 2 : 1 to 3 : 1 in contrast to a 1 : 1 ratio in commercial tallow (see Table 2). In this regard the subcutaneous beef fats resemble tallow from calves (*cf.* Dahl, 1957a). Again there is no difference in respect of this ratio between subcutaneous and perinephric sheep fats.

The high stability of subcutaneous beef fats against atmospheric oxidation as measured by their induction period (*cf.* Table 4) is remarkable. Stability tests were conducted on steam-rendered fats only, as solvent extraction has yielded fats of a very low stability (3–4 hr. induction periods). From the data shown in Table 4, it would appear that the induction periods are to some extent related to pressures used during the digestion, which accords with the earlier finding in this laboratory (Hartman and White, 1952). By removing some of the antioxidants formed during the digestion, alkali refining reduced the induction period which, nevertheless, compared favourably with that of commercial tallows.

TABLE 4—Induction Period of Steam Rendered Subcutaneous Beef Fats Before and After Alkali Refining

Sample	Steam Pressure During Digestion lb/sq. in.	Induction Period (hr)	
		Before Refining	After Refining
Commercial	Atmospheric	21	16
Commercial	75	45	22
N52	45	19	17

INDUSTRIAL APPLICATIONS

The potential economical value of subcutaneous beef fats for New Zealand is based on their low melting points, stability against oxidation, and their high ratio of palmitic to stearic acid. The two first-mentioned characteristics make them suitable as a substitute for "oleomargarine" i.e., the low melting portion of edible tallow which is used extensively in the local margarine and baking industries. Since the manufacture of oleomargarine is a time- and labour-consuming process, the alternative use of good quality subcutaneous fat seems to be advantageous.

Another market for subcutaneous fats from both oxen and sheep appears to be in the soap industry. The use of these low melting fats would increase the solubility of bar soaps and soap powders in water, improve their lathering properties, and ensure quicker rinsing. Finally, the ratio of palmitic to stearic acid in subcutaneous beef fats being higher than in hard tallows, makes them suitable material for the local stearic acid

industry where difficulties are sometimes experienced because of the poor crystallising properties of the fatty acid stock deficient in palmitic acid.

The total quantity of beef subcutaneous fat recoverable in New Zealand meat works would be in the vicinity of 1,000 tons per annum. The quantity of subcutaneous sheep fat is probably of a similar order. These could be supplemented by fats from brisket and similar parts of the carcase, which are often trimmed off from overweight animals. Brisket fats have, according to Dahl (1957) even higher iodine values than fats trimmed from the skins.

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THE REACTION BETWEEN PIE WOOL AND OXYGEN

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Summary

The reaction between pie wool and oxygen has been studied by measurements of rate of oxygen absorption and of behaviour in a standardised ignition test. It is shown that the reaction kinetics are similar to published data for the oxidation of unsaturated fatty acids, lending support to the view that spontaneous ignition of pie wool is due to atmospheric oxidation of unsaturated fats. Experiments demonstrate the effects of temperature, oxygen partial pressure, partial pressure of water vapour, presence of inhibitors and catalysts, distribution of fat, and area of surface presented by the wool. The order of reaction with respect to oxygen is discussed in relation to the order reported for other fat oxidation reactions. Analyses are presented for a range of pie wools. It is shown that a heating reaction can be produced by mixing unsaturated fatty acids with wool, and that this reaction is strongly catalysed by fat-soluble iron.

INTRODUCTION

When wool is removed from sheepskin pieces by the pie process (Wright, 1921) it becomes contaminated with subcutaneous fat. The large surface area of wool facilitates reaction of atmospheric oxygen with the fat, and Walker and Williamson (1957) have shown that heat generated by this reaction can cause spontaneous ignition of pie wool. It is desirable to know how this rate of heat generation is affected by such factors as temperature, reactant concentration, partial pressure of water vapour, presence of oxidation catalysts and inhibitors, etc. Such information is best obtained by measurements of rate of heat output under various conditions, but this would demand measurements of very low levels of heat output. For instance, if it be assumed that the standardised ignition test applied by Walker & Williamson (1957) was completely adiabatic, then the more reactive pie wools examined by these authors must have been generating heat at rates of about 10^{-2} cal/sec/g at temperatures around 110°c. To avoid the difficulties of measuring similar or even smaller quantities of heat, many investigators of suspected spontaneous ignition mechanisms have determined the effects of varying temperature, reactant concentration, etc., by measuring rates of reactant consumption or of product formation, or by applying a standardised ignition test. The simplicity of such experimental techniques facilitates examination of a wide range of samples, whilst the complexity of precise calorimetry has frequently limited its application in any investigation to a few samples or to only one (e.g. Burgoyne and Thomas, 1951). In choosing investigational methods other than calorimetry, the assumption must be made that heat output is related to the parameter

actually being measured, independently of variations of experimental conditions such as temperature, reactant concentration, etc.

Investigations of reaction kinetics have been described for direct gaseous oxidation of oleic or elaidic acids (with varying diene or triene concentrations) to elucidate such matters as the development of rancidity, the mechanism of biological oxidation, and the possibilities of spontaneous ignition. Although many investigators have been successful in using disappearance of double bonds to measure the effects of temperature and/or reactant concentration on oxidation of a pure fatty acid or its methyl ester (e.g. Henderson and Young, 1942; Mukherjee, 1950; and others), the iodine values determined by Burgoyne and Thomas (1951) throw doubt on the validity of using iodine values as a measure of reaction over wide temperature ranges. Further, the relatively high iodine value of the natural wool wax of cross-bred sheep (Walker and Williamson, 1957) renders iodine value of doubtful applicability to the present problem of oxidation rate of pie wool. Another widely used measure of reaction has been determination of peroxide values (e.g. Atherton and Hilditch, 1944; Gunstone and Hilditch, 1945, 1946), but similar criticisms would apply to this technique also (Mukherjee, 1950; Burgoyne and Thomas, 1951; Walker and Williamson, 1957). A study of the data reported by Hamilton and Olcott (1937) for oxidation of pure oleic acid and methyl oleate, throws further doubt on the applicability of iodine or peroxide values to this problem. A method better suited to measurement of the progress of reaction for kinetic investigations is absorption of oxygen. Burgoyne and Thomas (1951) established a correlation between absorption of oxygen by palm kernel fat spread on glass cloth, and the heat output of the reaction at one temperature (80°C). And Henderson and Young (1942) obtained consistent reaction rate data by measuring the oxygen absorption of well-stirred oleic acid at 80°C.

As regards application of a standardised ignition test, the well-known Mackey test (1896) has been modified by Kehren (1939) to investigate the effect of temperature on oxidation of a textile "oleine" spread on cotton, with indifferent success. The Mackey test has been used more successfully to investigate the effects of impurities such as iron, and the effects of pretreatment of the fat with hot air (e.g. Garner, 1936; Van Elteren, 1958). The test proposed by Walker and Williamson (1957) is better suited than the Mackey test to investigate reaction kinetics under various conditions of oxygen partial pressure and the presence of impurities, since the incoming gas is preheated, and the larger sample renders the course of combustion less dependent on order of reaction with respect to the unsaturated oil. It was therefore decided to use this test to investigate the reaction between oxygen and pie wool at 100°C and above; and to investigate the effect of temperature on the reaction by measurements of oxygen absorption.

Samples of pie wool were chosen for this investigation on the basis of their known reactivity, and are referred to by laboratory sample number. (These numbers are consistent with those quoted by Walker and Williamson, 1957). Analyses of all these pie wools are quoted in Table 1, some

TABLE 1—Heat-testing and Analysis of Selected Pie Wools

Sample Number	Moisture Content % on Dry Weight	Ether Extract % on Dry Weight	Reaction with Oxygen at 100° Wool Temperature Rise °C	Reaction with Oxygen at 100° Time Taken to Reach Maximum Temperature min.	Acid Value*	Iodine Value*	Saponification Value*	Lea Peroxide Value*	Ash %	Iron p.p.m.	Manganese p.p.m.	Nickel p.p.m.	Chromium p.p.m.	Zinc p.p.m.	Copper p.p.m.	Lead p.p.m.	Tin p.p.m.	Calcium p.p.m.	Magnesium p.p.m.
4	9.4	24.0	Fired	60	120	47	182	85	0.20	200	Neg	5	Neg	200	20	Neg	5	600	120
22	13.3	8.4	1.	230	25	28	116	—	0.73	500	100	Neg	Neg	300	70	50	Neg	2000	400
43	14.8	3.4	Fired	98	85	54	206	70	—	—	—	—	—	—	—	—	—	—	—
44	7.7	38.5	Fired	11	161	30	218	28	0.18	270	18	Neg	1	18	11	4	Neg	1100	18
66	13.3	11.4	Fired	202	82	—	—	39	—	—	—	—	—	—	—	—	—	—	—
71	14.7	5.3	Fired	155	—	—	—	—	0.37	800	3	4	3	100	—	7	1	600	300
72	9.7	28.5	Fired	29	70	32	—	—	—	—	—	—	—	—	—	—	—	—	—
78	9.3	16.4	Fired	67	41	—	—	23	—	—	—	—	—	—	—	—	—	—	—
81	10.7	29.6	Fired	35	129	23	212	13	0.26	150	8	Neg	Neg	50	10	5	Neg	1000	200
87	12.2	10.3	8	395	20	—	—	192	—	—	—	—	—	—	—	—	—	—	—
94	13.6	3.9	4	290	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
96	11.9	8.6	Fired	99	—	—	—	—	0.18	35	4	Neg	Neg	90	11	5	Neg	900	35
98	14.4	5.8	2	255	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
208	11.1	17.8	Fired	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

219	11.6	14.7	Fired	127	—	—	—	—	0.07	15	1	2	Neg	100	7	2	Neg	270	20
233	15.0	12.7	Fired	109	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
248	12.5	11.8	Fired	90	—	—	—	—	0.05	15	3	Neg	1	50	8	2	Neg	200	10
305	13.6	12.4	Fired	102	—	—	—	—	0.13	26	5	Neg	Neg	50	8	6	Neg	800	25
317	14.2	10.0	Fired	104	—	—	—	—	0.03	14	1	Neg	Neg	70	3	1	Neg	200	7
410	12.1	18.1	Fired	75	150	—	—	59	0.22	65	2	Neg	Neg	35	4	4	Neg	1300	35
436	13.3	18.2	48	365	—	—	—	—	0.50	200	4	1	Neg	150	10	13	Neg	1000	750
437	11.5	10.0	Fired	116	—	—	—	—	0.28	110	3	1	Neg	110	3	6	Neg	1100	300
438	12.3	10.3	Fired	234	—	—	—	—	0.09	35	13	Neg	Neg	26	5	5	Neg	520	13
446	9.8	21.3	Fired	19	135	28	209	7	0.40	160	2	Neg	Neg	40	6	12	Neg	2500	400
449	10.1	17.4	Fired	66	159	37	201	22	0.09	90	4	Neg	Neg	45	16	3	Neg	200	90
536	12.0	30.3	Fired	32	—	—	—	178	0.21	85	6	Neg	Neg	30	17	5	Neg	1300	35
614	11.1	23.0	Fired	41	—	—	—	75	0.67	130	70	1	Neg	25	40	7	Neg	4000	100
905	12.4	10.5	Fired	96	115	49	170	—	0.48	500	10	10	Neg	200	35	10	10	2500	600
2007	11.1	10.3	Fired	46	116	—	—	147	0.55	500	4	Neg	1	60	50	30	Neg	—	—
2017	9.7	22.8	Fired	16	—	—	—	—	—	1000	—	—	—	—	—	—	—	—	—
2019	15.8	5.9	28	145	—	—	—	—	0.97	—	80	1	Neg	190	100	1	1	2500	1800
2029	13.5	18.4	Fired	102	—	—	—	—	—	—	—	Neg	—	—	—	—	—	—	—
2037	13.9	12.4	Fired	33	—	—	—	—	0.16	500	80	Neg	Neg	30	25	Neg	Neg	320	130

NOTE: * = B.S. 684:1950.   p.p.m. = parts per million.   Neg = negligible.   — = not determined.

figures reported by Walker and Williamson (1957) being repeated here for convenience. As before (Walker and Williamson, 1957) the diethyl ether extract is referred to as "fat", and the "firing point" in the ignition test is taken as 180°c. The metal compounds soluble in ether were analysed spectrographically, and no significant amounts of catalytic metals other than those quoted were detected. Pie wools which had been involved in fires in commerce are samples 72, 81, 614, and 2017.

(1) INDUCTION PERIOD

An extensive literature exists on the phenomenon of an induction period in oxidation of unsaturated fatty acids. More than five hundred different samples of pie wool have now been examined by the standardised ignition test described by Walker and Williamson (1957), and these results have been studied for evidence of an induction period. The bulk of these samples showed time/temperature curves of similar shape, differing mainly in steepness of rise. However, some variations in the shape of the time/temperature curve were observed in this programme, and graphs of
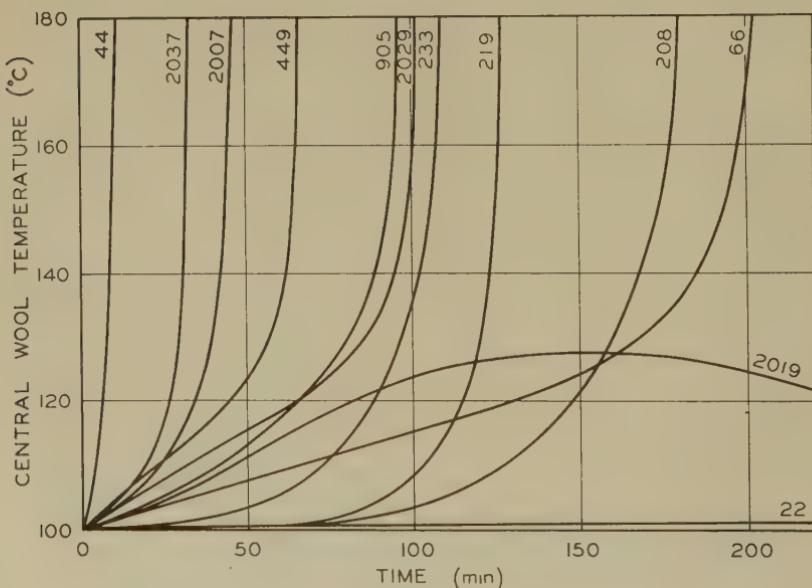
FIG. 1

selected pie wools showing a range of different behaviours are shown in Fig. 1. These differences are probably due more to variations in reactivity, total quantity of fat present, and presence of diluents in the fat, than to induction period. However, samples 208 and 219 probably exemplify a true induction period. A behaviour ranging between that shown by samples 233 and 449 is typical of most unscoured hot-tank pie wools.

In the measurements of oxygen absorption by pie wools (described

under *2, 3 & 4* below) no induction period was noticed; but this may be due to the relatively small number of wools examined, and to the fact that some preheating in air or oxygen was always carried out before the initial readings.

(2) RATIO OF CARBON DIOXIDE EVOLVED TO OXGEN ABSORBED

Many investigators of low temperature chemical oxidations have measured the molecular ratio of CO_2 evolved to O_2 absorbed. This is normally well below unity; and in addition to the fact that it is often typical of the reaction, a knowledge of its magnitude is useful in interpreting contraction of oxygen gas in terms of oxygen actually absorbed. Experimental techniques have often used a static volume of oxygen gas, but unless elaborate precautions are taken to keep this gas purified from gaseous reaction products (e.g. Ellis, 1932), these will build up and affect the reaction rate. A technique of continuous ventilation was therefore chosen for the present work. A constant rate of air flow was maintained over a sample of pie wool, and both the inflowing air and the outflowing gas were analysed. By assuming that nitrogen is unaffected by the reaction, the nitrogen content of air can be used as an internal standard to calculate absorption of oxygen.

100 g samples of pie wool (air-dry weight) were packed into $1\frac{1}{2}$ l. round glass flasks modified to provide a gas inlet opposite the exit. The flasks were immersed in a water-glycerine thermostat at 100°c, and the wool dried in a stream of dry carbon dioxide gas at 1·55 ml/sec (s.t.p.), passed through a heat exchanger in the thermostat. When the wool was dry, the thermostat was lowered to working temperature ($\pm0\cdot1$°c) and the incoming gas changed to dry air at the same flow rate. This dry air was purchased in a cylinder and analysed in an Orsat apparatus. It contained no carbon dioxide, and 21·0% oxygen. Although water would be formed as a reaction product, it could not appear in the gas phase since it would be absorbed by the dry wool (W.I.R.A., 1955). Two pie wools were tested in this way, analytical gas samples being drawn late in the day when conditions had stabilised. Overnight the wool samples were ventilated with dry commercial nitrogen (0·2% oxygen) to effectively halt the reaction. Measurements were made at two temperatures, reverting again to the initial temperature at the end of the experiment. Experimental data and calculated ratios of CO_2 evolved to O_2 absorbed are quoted in Table 2, together with estimates of the total O_2 absorption per g dry wool at the time of measurement. In interpreting this last figure, the fat content (Table 1) must also be considered. Wool at the centre of the flasks in these experiments would be considerably hotter than the thermostat bath (say 3°–5°c), particularly at the higher temperature.

Ellis (1932, 1936, 1950) investigated the late stages of catalysed oxidation of elaidic acid (200×10^{-3} g O_2/g fat), and found ratios of CO_2 evolved to O_2 absorbed ranging from 0·20 to 0·25. Burgoyne and Thomas, (1951) investigated the oxidation of palm kernel fat, and although they found lower ratios in early stages of the reaction, in later stages (64×10^{-3} g O_2/g fat) the ratio rose to 0·25. Both these reactions were

considered to be attack by oxygen at the double bond of elaidic and/or oleic acids. They both show close similarity to the present ratios in Table 2, provided the two ratios based on very low CO_2 contents are disregarded.

(3) Effect of Temperature

Rate of reaction was determined by measuring the rate of gas contraction in a closed vessel containing pie wool, held in a thermostat. This procedure has defects, first that the wool cannot be brought to a uniform temperature because of the heat of reaction; and second that contraction will not be a direct measure of oxygen absorbed, because of the presence of gaseous reaction products. The products of similar reactions have been investigated (Ellis, 1950; Burgoyne and Thomas, 1951), and found to be about 20% carbon dioxide and 10% water (percentages on the oxygen absorbed). Examination of the water relationships of wool (W.I.R.A., 1955) shows that the presence of dry wool in this experiment ensures that a negligible amount of water vapour would appear in the system. Provided the two experimental temperatures are not too far apart, the ratio of CO_2 evolved to O_2 absorbed will remain relatively constant at any one stage in the reaction. If it can be assumed that oxygen absorption is related to heat output, this technique offers a convenient measure of reaction rate.

Pie wool (200 g air-dry weight) was packed into a 3 l. round flask fitted with a central thermometer, and with gas inlet and outlet tubes, and immersed in a water thermostat ($\pm 0.05°C$). A stream of dry carbon dioxide was passed through the wool until it reached thermostat temperature (indicating dryness), and the gas stream was then changed to dry oxygen (99.5% pure), which was passed long enough to sweep out the carbon dioxide. The reaction flask was sealed off and gas contraction measured by a gas burette, holding the pressure equal to atmospheric by manual adjustment of an acidified saline confining fluid, using a cathetometer. Between measurements (overnight) the flask was swept with dry commercial nitrogen (containing 0.2% oxygen) to effectively halt the reaction, and to avoid the difficulties experienced by Hilditch and Sleightholme (1932) due to accumulation of reaction products, and also the difficulties experienced by Thompson (1928) due to release of volatile constituents from the fat. This technique has an advantage over that used by Burgoyne and Thomas (1951) since oxygen partial pressure is reasonably constant throughout the measurement.

Two pie wools 4 and 81 (see Table 1) were tested in this way at approximately 63° and 73°C. Gas contractions (ranging from 20 to 100 ml) were measured over periods of a few hours, and corrected for small variations of wool temperature and of atmospheric pressure, to s.t.p. The centre of the wool was hotter than thermostat temperature by up to 1°C (depending on reaction rate), and the thermostat temperature was therefore adjusted to bring the mean temperature of the wool close to the desired temperatures of 63° or 73°C. From the finally determined temperature coefficient the reaction rate was then corrected to standard temperatures of 63° and 73°C, this correction always being less than $\frac{1}{2}$°C.

TABLE 2—Ratios of CO₂ Evolved to O₂ Absorbed by Pie Wool

Thermostat Temperature °C	Pie Wool No. 4				Pie Wool No. 72			
	Estimated Total Oxygen Absorption 10⁻³ g/g Dry Wool	Analysis of Exit Gas % by Volume CO₂	O₂	Ratio	Estimated Total Oxygen Absorption 10⁻³ g/g Dry Wool	Analysis of Exit Gas % by Volume CO₂	O₂	Ratio
93.8	15	0.2	20.0	(0.16)	32	0.6	18.6	0.21
107.4	31	1.0	16.3	0.22	84	4.0	8.2	0.26
107.4	38	1.2	15.6	0.18	102	2.7	12.1	0.26
93.8	61	0.2	19.8	(0.14)	135	0.5	19.3	0.25

Each wool showed a marked reduction in reaction rate during the experiment, demonstrating that order of reaction with respect to pie wool is greater than zero. This diminishing reaction rate makes a direct comparison difficult between rates at the two temperatures. By measuring contraction rates at two temperatures, and then allowing the reaction to progress before making further measurements, the effect of order of reaction can be recognised and minimised. In Figs 2 and 3 these measured contraction rates are plotted on a log scale against estimates of the total absorption of oxygen by the wool (on a linear scale). If it be assumed that the temperature coefficient is constant at all stages of the reaction, it is permissible to draw parallel lines (not necessarily straight) on such a scale, yielding ratios between rates at the two temperatures. The lack of fit is assumed to be due to experimental error, since the two samples were together in the same thermostat. It will be noticed that the alternative technique of averaging the rate at each temperature and then calculating ratios, would give similar results. At a mean temperature of 68°C the temperature interval necessary to change the reaction rate by a factor of two is seen to be 9°C for sample 4, and 8°C for sample 81. If it is assumed that the influence of temperature on the reaction is in accordance with the Arrhenius Law, then conventional calculation of the energy of activation yields figures of 17 and 20 kcal per mole oxygen for 4 and 81 respectively.

Burgoyne and Thomas (1951) investigated the rate of oxygen absorption by palm kernel fat spread on glass cloth, and measured a constant temperature interval of 10·1°C to double the rate over the range 50°–160°C. The Arrhenius Equation was not obeyed. Gross and Robertson (1958) measured temperature coefficients of heat generation of a series of oils spread on cotton gauze and exposed to air in an adiabatic apparatus. They examined sperm, olive, castor, and neatsfoot oils, and found temperature intervals to double the reaction rate ranging from 8·4° to 13·8°C, over temperatures varying between 115° and 170°C. Gunstone and Hilditch (1945) investigated the rate of peroxidation of pure methyl oleate over the temperature range 20°–130°C. Although their figure for 20°C is anomalous, their published results show that between 50° and 120°C the effect of temperature on the reaction is in accordance with the Arrhenius Law, with an energy of activation of 17·3 kcal per mole oxygen. At a temperature of 68°C this corresponds to a temperature interval of 9·3°C for the rate to double. The temperature coefficients of reaction here measured for pie wool at 68°C show close similarity with all these published figures for addition of oxygen to the double bonds of unsaturated fats, suggesting that a similar reaction is involved. The similarity to the peroxidation of methyl oleate (Gunstone and Hilditch, 1945) may be explained by the care these authors took to see that the stage was not reached where peroxide values cease to increase and begin to decrease; and by the demonstration by Henderson and Young (1942) that peroxide content is related to oxygen absorption for pure oleic acid at early stages in the oxidation.

Spontaneous fires are known to start in commerce at temperatures below 63°C, and it is of interest to investigate the reaction rate at lower temperatures. Walker and Williamson (1957) described an adiabatic apparatus in which they raised a sample of pie wool to combustion from a temperature

RATE OF GAS CONTRACTION 10^{-6} ml/sec/g dry wool (s.t.p.)

ESTIMATED TOTAL ABSORPTION OF OXYGEN
10^{-3} g O_2/g dry wool
o = 73° C
⊗ = 63° C

FIG. 2

RATE OF GAS CONTRACTION 10^{-6} ml/sec/g dry wool (s.t.p.)

ESTIMATED TOTAL ABSORPTION OF OXYGEN
10^{-3} g O_2/g dry wool
o = 73° C
⊗ = 63° C
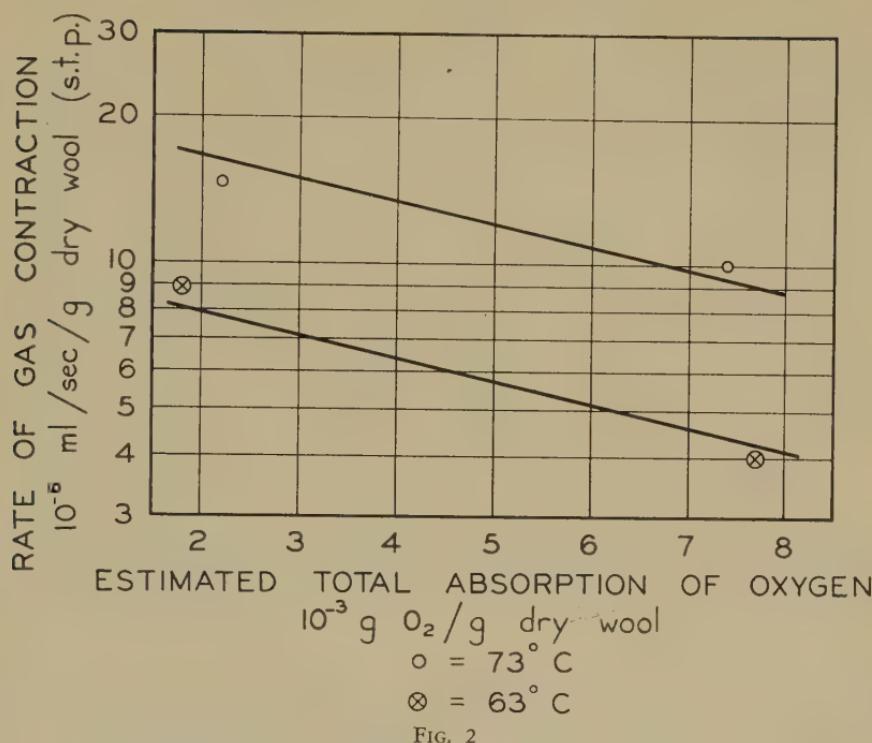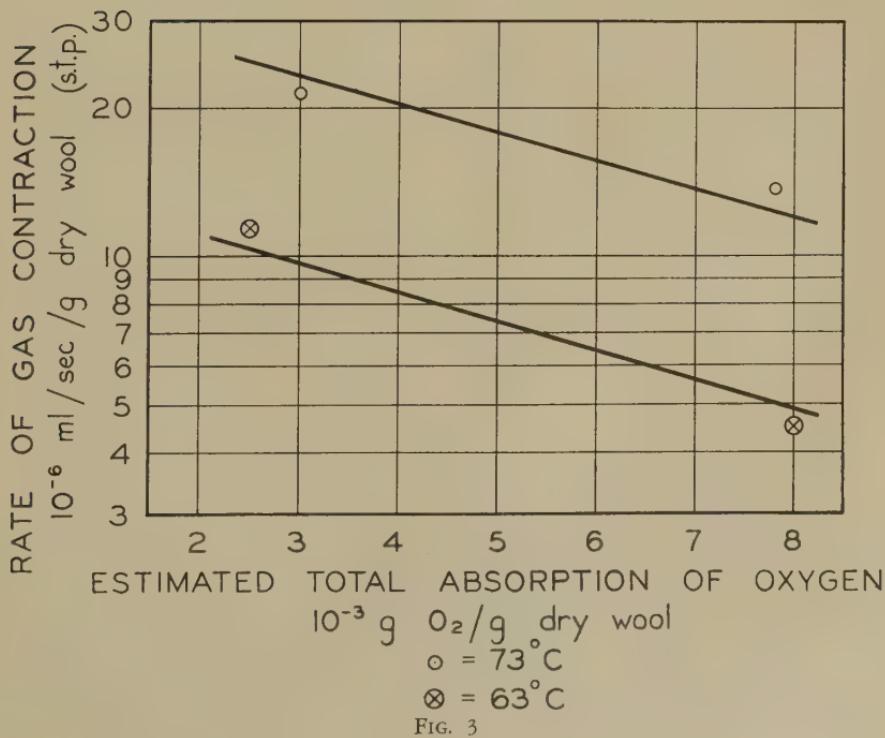
FIG. 3

of 55°C. In several trials it was not found possible to produce adequate self-heating of pie wool in air in this apparatus at temperatures below 52°C. One possible method of investigating the reaction at lower temperatures is suggested by the falling off of gas contraction rate in the absorption experiments described above. To further investigate this effect, a typical pie wool (449) was held for 390 hours in air at a temperature of 60°C, and then subjected to the normal ignition test. It showed an unusual behaviour in this test, giving a very long slow temperature rise; but failed to take fire, and finally reached a peak temperature rise of 21°C in 680 minutes. If the measured temperature coefficients applied down to say 17°C, storage at room temperatures for between two and four years should cause a similar effect. Yet Walker and Williamson (1957) described repeat tests carried out over a five-year period on samples of pie wool stored at normal air temperatures, and reported coefficients of variation no greater than 20%. This ignition test is admittedly not related in a simple manner to reactivity, and any test of this type probably exercises a maximum degree of discrimination at some characteristic critical reaction rate (superimposed on the order of reaction). In this context Thompson (1927) has remarked that the Mackey test (1896) divides oils sharply into two classes, although no such division exists in practice. Walker and Williamson's test (1957) probably does the same.

Ignition tests (Walker and Williamson, 1957) were carried out on a number of pie wools over a ten-year period, and the results are shown in Table 3. These samples were given normal storage in the laboratory at temperatures varying seasonally between say 10° and 24°C. It can be seen that the more reactive samples appear to be less affected by storage; but this difference may reflect the characteristics of the test rather than a real difference in behaviour during storage. Although not particularly quantitative, this trial suggests that the measured temperature coefficient does not always apply right down to room temperatures, but that rates at room temperature are much lower than might be expected. This behaviour is the reverse of that noticed for peroxidation of methyl oleate by Gunstone and Hilditch (1945). It may well be that an anomaly would occur at the m.p. of the fat (pie fats are normally solid at room temperature). In this connection Ellis (1932) has pointed out that although catalysed oleic and elaidic acids oxidise at comparable rates at temperatures above 50°C, catalysed elaidic acid does not absorb oxygen at temperatures below its m.p.

(4) Effect of Oxygen Partial Pressure

The effect of variations in oxygen partial pressure was investigated by carrying out gas contraction rate measurements similar to (3) above using various mixtures of dry oxygen and nitrogen purchased in cylinders, and analysed. In addition to the corrections described under (3), the lower values of oxygen content necessitated small corrections for diminution of oxygen partial pressure during each experiment. Since the results of these experiments yielded ratios of reaction rates at different oxygen partial pressures, these ratios were used to make the corrections. As before, if it be assumed that the effect of oxygen partial pressure is the same at all stages of the

TABLE 3—Ignition Tests on Pie Wools Stored at Room Temperature

Sample No.	Storage Time (Years)					
	0	2	4	5½	8	9½
44	Fired in 11 min					Fired in 30 min
536	Fired in 32 min					Fired in 90 min
81	Fired in 35 min					Fired in 76 min
614	Fired in 41 min			Fired in 72 min		Fired in 119 min
410	Fired in 75 min	Fired in 113 min	Fired in 249 min		Fired in 335 min	
248	Fired in 90 min			Heated 4°c in 200 min		
43	Fired in 98 min	Fired in 120 min				
96	Fired in 99 min			Heated 7°c in 180 min		
305	Fired in 102 min		Heated 6°c in 150 min			
317	Fired in 104 min		Heated 3°c in 250 min			

reaction, the contraction rates can be plotted against estimates of total oxygen absorption on a log linear scale, and parallel lines (not necessarily straight) can be drawn through the various oxygen concentrations. These are shown for samples 81 and 614 in Figs 4 and 5. The two flasks were mounted in the same thermostat, and readings were taken at the same times, so corrections for slight variations in temperature and atmospheric pressure would affect both wools equally. Figs 4 and 5 demonstrate definite differences between the two wools in their response to oxygen partial pressure.

The effect of oxygen partial pressure on the rate of oxygen absorption by pure oleic acid has been investigated by Henderson and Young (1942). They worked at normal atmospheric pressure, and used mixtures of oxygen and nitrogen varying between air and pure oxygen. They claimed to establish an order of reaction with respect to oxygen of $n = \frac{1}{2}$, but ignored the fact that their suggested rate equation postulates that a considerable reaction would take place with no oxygen present. This error was due to
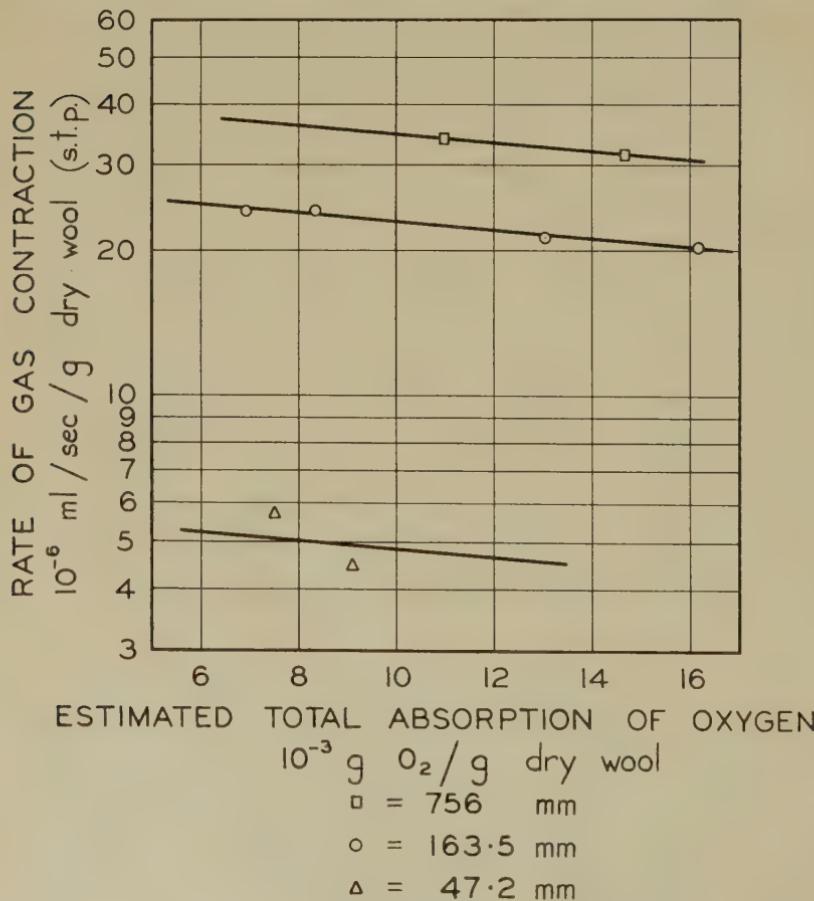
Fig. 4

failure to carry out experiments with mixtures containing less oxygen than air contains, and failure to recognise that no action would take place in pure nitrogen. Henderson and Young (1942) measured a factor of 2·0 in reactivity between oxygen and air. Burgoyne and Thomas (1951) measured the rate of absorption of oxygen by palm kernel fat dispersed on glass cloth at various initial oxygen pressures. Although these authors worked at constant volume and therefore allowed oxygen partial pressure to vary throughout each experiment, it is possible to estimate the true effect of oxygen partial pressure by considering only the early stages of each of their experiments. They measured a factor in reactivity of 1·8 between oxygen and air. Their experiments at lower oxygen partial pressures are unreliable because of changes in pressure during the experiment. Burgoyne and Thomas (1951) suggested a rate equation for the higher oxygen pressures, of a form similar to that proposed by Henderson and Young (1942), with

$$\square = 756 \quad mm$$
$$\odot = 163·5 \ mm$$
$$\triangle = 47·2 \ mm$$
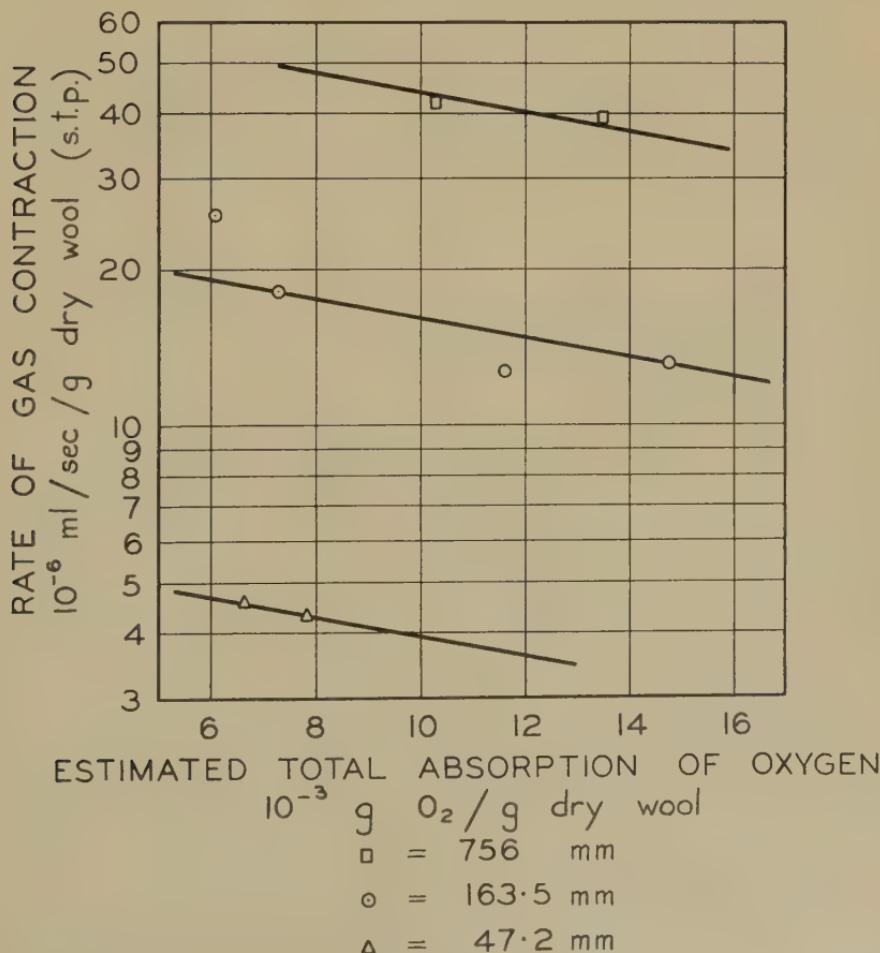
FIG. 5

an order of reaction with respect to oxygen of $n = 0.72$. However, they pointed out that this equation would not hold at low oxygen pressures, and recognised (and measured) that the reaction rate falls to zero as the oxygen partial pressure approaches zero. The similarity of the above two ratios for reactivities in air and oxygen with the ratios of 1.5 and 2.7 in Figs 4 & 5 again suggests that oxidation of the fat in pie wool is proceeding by addition of oxygen to the double bond of oleic acid.

Further evidence on the effect of oxygen partial pressure was obtained by modifying the ignition test proposed by Walker and Williamson (1957) to use dry air instead of dry oxygen. Four pie wools were tested with air in this way, and these results may be compared with the standard figures in Table 1,

Sample 2017 — in air, fired in 34 min.
Sample   81 — in air, heated 50°C in 150 min.
Sample   536 — in air, heated 60°C in 192 min.
Sample   449 — in air, heated 31°C in 630 min.

Although this approach is less quantitative than oxygen absorption measurements, it has the advantage of being directly related to the heat output of the reaction. The results are consistent with the above conclusion that the reaction rate is reduced by a factor of about two in changing from oxygen to air.

(5) Effect of Water

It has sometimes been suggested that traces of water vapour are necessary to catalyse oxidation. No sign of such an effect could be detected in experiments in which Walker and Williamson's test (1957) was carried out with samples dried up to 10 days with the incoming gas passed over dry magnesium perchlorate.

However, the influence of water under damper conditions is also relevant to the problem. The normal test is carried out with dry gas to avoid the considerable thermal effects which would accrue from movements of the equilibrium water in wool as the temperature changes. Walker and Williamson (1957) demonstrated that bales of wet wool can become hot, and our own (unpublished) experiments show that damp wool (RH < 100%) will also become hot. This heating is due to microbiological action. It is important to know the effect of water on the oxidation reaction, to assess what part microbiological heating of wet or damp wool might play in heating bales of pie wool to a stage where chemical oxidation could begin. This was investigated by carrying out Walker and Williamson's test (1957) with all incoming gases humidified to a definite dew point by leading them through a water-filled bubbling train in a separate water thermostat. To avoid condensation in the gas pipe between this thermostat and the boiling water oven, it was electrically heated and lagged.

Table 4 shows results of tests on a number of pie wools, carried out in this manner at various dew points. The higher dew points cause an appreciable equilibrium moisture content in the wool, and since some of this moisture would be lost as the temperature rose, the heat absorption so repre-

TABLE 4—Ignition Tests Using Oxygen at Various Dew Points

Sample No.	Dry	Oxygen Dew Point							
	Dry	25°C	35°C	45°C	50°C	55°C	60°C	70°C	80°C
2017	Fired in 16 min				Fired in 27 min		Fired in 32 min	Fired in 43 min	Fired in 61 min
81	Fired in 35 min					Fired in 176 min		Fired in 324 min	
449	Fired in 66 min			Fired in 158 min				Fired in 260 min	
410	Fired in 75 min	Fired in 93 min	Fired in 131 min	Fired in 147 min		Fired in 305 min			
437	Fired in 116 min			Fired in 305 min			Heated 11°C in 375 min		
438	Fired in 234 min			Heated 6°C in 237 min			Heated 7°C in 235 min		
436	Heated 48°C in 365 min			Heated 9°C in 240 min			Heated 7°C in 225 min		

sented would delay the temperature rise. Moreover, the higher humidities represent a considerable aqueous tension, resulting in a lowered oxygen partial pressure. For example, the incoming reactant gas at a dew point of 70°C would contain only 69% oxygen by volume, and this too would slightly inhibit the reaction. This effect is of course real, since it would operate also in any practical case of combustion of wet wool in air, but it acts to mask the results of the present experiments. Although these experiments are relatively crude, they show that despite these two effects the oxidation of pie wool is definitely inhibited by the presence of moisture.

General opinion in the literature is that the presence of moisture will not greatly affect the rate of oxidation of unsaturated fatty acids. Thompson (1928) studied atmospheric oxidation at 100°C of linseed oil spread on cotton waste, and stated that the presence of moisture actually increased the rate of heat production. Ellis (1936) studied the absorption of oxygen by catalysed elaidic acid dispersed on fine sand at temperatures between 50° and 80°C, and found that the presence of water vapour did not appreciably affect the rate of oxidation, even in atmospheres saturated at the temperature of the experiment. Van Elteren (1958) studied the generation of heat caused by oxidation of a catalysed textile oleine, and found that the lowest temperature at which heating first became apparent was relatively uninfluenced by the presence of water or water vapour. The rate of heating was decreased by the presence of water, but not to the same extent as in the present experiments.

Microbiological heating of wet wool must therefore be considered as a possible trigger mechanism to preheat bales of wool to a point where oxidation could commence, since these experiments show that oxidation of pie fat can take place in the presence of considerable moisture.

(6) PRESENCE OF DIENE AND TRIENE ACIDS

Hilditch (1947) has stated that sheep body fats do not contain linoleic or linolenic acids, although about 4% ($\pm$ 1%) of an octadecadienoic acid is present. It was considered that variations in this content of diene and/or triene acid might explain the differences in oxidation behaviour remarked by Walker and Williamson (1957) between pie wools of similar fat analysis.

Six pie wools were selected and their fat analysed for diene and triene acids. This showed,

Sample 44 – 0·3% diene, 2·6% triene
Sample 78 – 1·5% diene, 1·5% triene
Sample 81 – 0·2% diene, 2·4% triene
Sample 87 – 1·9% diene, 1·7% triene
Sample 94 – 0·9% diene, 1·6% triene
Sample 98 – 0·3% diene, 1·6% triene

The reason these figures are below those quoted by Hilditch is probably the presence of an indeterminate amount of wool wax in each pie wool. A comparison with Table 1 does not show any clear relationship between reactivity with oxygen, and the diene and triene acid contents.

(7) REMOVAL OF FAT FROM PIE WOOL

It is known that conventional Soxhletting with diethyl ether will not remove all greasy material from a sample of wool, and that subsequent Soxhlet extraction with ethyl alcohol can sometimes remove up to several per cent more fat. It was considered that this might explain Walker & Williamson's (1957) failure to establish a correlation between firing and fat content; and also might explain these authors' failure to reduce the reaction between pie wool and oxygen to zero by extracting the wool with ether.

A sample of very reactive pie wool (446) was Soxhletted with diethyl ether until no further fat was removed, and found to have a fat content of 21·3% on the dry weight of the greasy sample. Duplicate samples thus extracted were subjected to Walker and Williamson's test (1957), and found to heat only 2°C, rising to this maximum temperature in 6 hours. An extraction with ethyl alcohol was carried out on further ether-extracted material, and was found to yield 5·6% fatty material (expressed on the dry weight of the original sample). Duplicate samples so extracted heated only 1°C in the test, rising to this maximum temperature in 5 hours.

It was considered possible that the fat subsequently alcohol-extracted might have a content of catalytic metals different from that of the fat originally extracted with ether. To check this, four pie wools were selected and ether-extracted, and then alcohol-extracted. The spectrographic analyses of these alcohol extracts are shown in Table 5. No significant amounts of other catalytic metals were detected. It is apparent that these figures do not significantly alter the analyses in Table 1.

(8) DISTRIBUTION OF FAT IN PIE WOOL

As Frank-Kamenetskiĭ (1955) has pointed out, the rate of a two phase gas/solid reaction may be controlled either by true chemical kinetics, or by the rate of gaseous diffusion. One factor deciding which of these becomes the rate-controlling mechanism is the area presented by the fat to the reactant oxygen. This area could be influenced by the state of distribution of the fat on pie wool; and one method of investigating if this is the case is to re-distribute the fat, and compare its ignition behaviour after this treatment with its original behaviour.

When sample 536 was immersed in water at 35°C and allowed to dry in the laboratory air, it showed a peroxide value of 211 and fired in the test in 22 minutes. Two further samples were similarly briefly immersed in water at 35°C and dried for seven days in air at 35°C, and showed peroxide values of 82 and 94, and fired in 22 and 25 minutes respectively. Further samples with 0·01 and 0·1 g/l of a wetting agent (Lissapol N) added to the warm water showed peroxide values 76 and 96, and firing times 28 and 29 minutes. As can be seen in Table 1 these treatments have caused a significant drop in peroxide values (originally 178), and a barely significant drop in firing time (originally 32 minutes).

Next a 200 g sample of 449 was immersed in a mixture of 200 ml diethyl ether and 500 ml petroleum ether (BP < 40°C) in a large glass

TABLE 5—Analysis of Ether-Extracted Pie Wool Residues

Original Sample Number	Alcohol Extract % on Original Dry Weight	Ash %	Analysis of Alcohol Extract									
			Iron p.p.m.	Manganese p.p.m.	Nickel p.p.m.	Chromium p.p.m.	Zinc p.p.m.	Copper p.p.m.	Lead p.p.m.	Tin p.p.m.	Calcium p.p.m.	Magnesium p.p.m.
71	4.8	19.5	60	12	10	Neg	300	—	10	Neg	40,000	3000
446	5.6	14.1	30	30	10	4	300	10	40	5	14,000	3000
2019	4.4	31.1	190	25	30	Neg	2500	500	30	10	30,000	3000
2037	4.2	6.9	200	40	10	Neg	70	70	100	Neg	8000	500

basin. The wool was well kneaded with the hands (rubber gloves) and turned over and over until all solvent had evaporated. Three such samples gave firing times of 71, 75 and 86 minutes (originally 66 minutes). Four further samples were treated similarly with ether and petroleum ether, and then immersed in water at 50°C for 3 minutes, and dried for two days in air at 35°C. These samples fired in 58, 63, 75, and 102 minutes respectively. In a similar manner wool 410 was treated with ether and petroleum ether, and then fired in 87 minutes (see (9) below). This is not significantly different from its original time of 75 minutes.

There is some slight evidence from these (and other) experiments that the reactivity of pie wool is slightly increased by wetting with water and drying immediately prior to test. Of somewhat more interest are the results of experiments where the fat was dissolved from the wool in the mixed solvent, and re-distributed. As can be seen this treatment had a barely measurable effect on reactivity. It is thus evident that the dispersion of reactive fat on pie wool must approximate to an even distribution of fat over the surface of the fibre. Moreover, the solvent evaporation technique here demonstrated offers an opportunity to distribute various materials on wool, and compare their behaviour with pie wool.

(9) Effect of Oxidation Inhibitors

An extensive literature exists on the influence of oxidation inhibitors (Moureu & Dufraisse, 1922), on the oxidation of unsaturated fats. It is of interest to see if inhibitors exert the same influence on the pie wool reaction.

A 200 g sample of 410 was well mixed with a mixture of 200 ml diethyl ether and 500 ml petroleum ether (BP < 40°C) to which had been added some β naphthol. This was mixed as described above until all solvent had evaporated, and then tested for ignition (Walker and Williamson, 1957). Firing times were as follows (β naphthol expressed on dry weight of wool),

    No β naphthol – fired in 87 minutes
    0·0004% β naphthol – fired in 84 minutes
    0·004% β naphthol – fired in 225 minutes
    0·04% β naphthol – fired in 365 minutes
    0·4% β naphthol – failed to fire during 24 hours testing.

These experiments show that in this pie wool reaction the inhibitor behaves normally; that is, the induction period is extended until all the inhibitor is consumed, and normal oxidation then takes place (Moureu & Dufraisse, 1922). Such a mechanism would not provide an ideal solution to the commercial problem of spontaneous ignition since it prevents (or considerably delays) the slow decay of reactivity at room temperature demonstrated in Table 3. Many authors have carried out experiments demonstrating that this effect can even increase the fire hazard under certain conditions (e.g. Van Elteren, 1958).

(10) OXIDATION OF OLEIC ACID AND TRIOLEIN

Walker and Williamson (1957) demonstrated that the fire hazard of pie wool is associated with fat content, but failed to relate it to analyses of the fat. They found that natural wool wax has no ignition hazard. Hilditch (1947) describes the constituents likely to exist in mutton tallow, the most reactive of which is oleic acid. Since it is not clear from the data of Table 1 and from the data presented by Walker and Williamson (1957) whether analyses have explained the reactivity of various pie wools, a synthetic approach was tried by using the solvent technique described under (8) above to distribute relatively pure fats on wool.

A sample of sheared wool was obtained having a fibre diameter comparable with that of typical pie wool. This wool was scoured with soap solution, and Soxhlet extracted with acetone. The mean fibre diameter (at 50% RH) was 29·4 microns (B.S. 3183:1959). The ignition hazard of oleic acid was investigated by dissolving it in ether/petroleum ether, and mixing this solution with the clean wool until all solvent had evaporated. The oleic acid used was BDH "Laboratory Reagent" grade, and no attempt was made to purify it. A sample of M & B "Laboratory Chemical" oleic acid purchased 8 years earlier showed very similar firing times. The various concentrations of oleic acid (expressed on the dry weight of the final composite wool sample) showed firing times as follows (duplicate samples),

No　　oleic acid – Negligible heating ($< 1°c$); ditto.
2·9%　oleic acid – Heated 10°C in 252 min; heated 12°C in 237 min.
5·6%　oleic acid – Fired in 151 min; heated 48°C in 175 min.
8·2%　oleic acid – Fired in 83 min; fired in 89min.
10·6%　oleic acid – Fired in 70 min; fired in 73 min.
12·9%　oleic acid – Fired in 48 min; fired in 50 min.
15·1%　oleic acid – Fired in 43 min; fired in 42 min.
19·2%　oleic acid – Fired in 37 min; fired in 38 min.

Some of this oleic acid was esterified to form triolein (glycerol trioleate), and this was similarly tested,

10·5%　triolein – Fired in 232 min.
14·0%　triolein – Fired in 185 min.

The molecular weight of triolein is only 10% greater than three oleic acid molecules, so this increase in firing time represents a reduction in reactivity. This relationship in oxidation rate between fatty acids and their glycerides has been found by other investigators also.

With the experiments using oleic acid in the concentration range 8·2% to 19·2%, the time taken to "fire" appears inversely related to concentration. This suggests either that the test conditions are reasonably adiabatic over this time range, or that heat losses are proportional to the time taken to rise in temperature. A rough approximation to the heat output might therefore be obtained by multiplying the rate of temperature rise by the specific heat. The two complications of a possible unknown induction period and of the fact that the temperature is changing throughout the experiment,

can be eliminated by considering the rate of temperature rise at say 110°C. Accordingly, the time/temperature curves for concentrations of oleic acid in the range 8·2% to 19·2% were plotted and tangents drawn at 110°C. At this temperature these showed average rates of rise of: 8·2% – 0·0115°C/sec; 10·6% – 0.0143°C/sec; 12·9% – 0.0173°C/sec; 15·1% – 0·0227°C/sec; 19·2% – 0·0299°C/sec. Accurate figures for specific heats of the two components are not known, so assuming a value of 0·36 cal/g/°C for all combinations, these figures respectively convert to 0·050, 0·049, 0·048, 0·054, and 0·056 cal/sec/g oleic acid (in oxygen at 110°C). Since calorimetric data for the oxidation of oleic acid is not available, these figures may be compared with the heat output of olive oil dispersed on cotton gauze as measured by Gross & Robertson (1958). Calculation from their data gives a figure of 0·012 cal/sec/g olive oil exposed to air at 110°C. It is obvious that in oxygen the figure would be about half the present results. The olive oil was a glyceride and therefore should be less reactive than oleic acid; although this might be partly compensated by the possible presence of a greater proportion of linoleates in olive oil than in the oleic acid.

(11) OXIDATION OF ELAIDIC ACID

The trans isomer of oleic acid (elaidic acid) is present in unknown amount in pie fats, since bacterial processes during pieing might cause cis/trans transformations. The behaviour of elaidic acid in the ignition test was investigated to see if cis/trans isomerism of this type could be responsible for the unexplained differences in reactivity between pie wools reported by Walker and Williamson (1957). Elaidic acid (L. Light & Coy) was mixed with acetone-extracted wool as described in (10) above. Duplicate ignition tests then showed,

5·6% elaidic acid – Fired in 208 min; Fired in 225 min.

10.6% elaidic acid – Fired in 161 min; Fired in 128 min.

It is thus seen that the elaidic acid was not as reactive as oleic. A minor part of this difference may be due to the difficulties of dispersing a crystalline solid over wool rather than an oil, since some of it inevitably powders off during manipulation. It is apparent that variations in reactivity between pie wools cannot be explained by postulating conversion of oleic acid into elaidic during pieing. It may be noted that Ellis (1936) has stated that catalysed oleic and elaidic acids absorb oxygen at comparable rates at temperatures above 45°C.

(12) OXIDATION OF STEARIC AND PALMITIC ACIDS

Stearic and palmitic acids (BDH "Laboratory Reagent" grades) were spread on wool as in (10) above. Again these crystalline materials were difficult to retain on the wool fibres without minor losses. Duplicate ignition tests showed,

24·8% stearic acid – Heated 1°C in 470 min; Heated 2°C in 460 min.

24·8% palmitic acid – Heated 3°C in 500 min; Heated 3°C in 700 min.

This demonstrates (as might be expected) that these saturated fatty acids show a negligible tendency to heat in oxygen at 100°C. The very slight heating here demonstrated may be related to the known slight absorption of oxygen by pure stearic acid at 100°C (Briggs & Thomas, 1939). Mixed saturated and unsaturated acids were next spread on wool to see if this would increase the reaction rate of either acid. Ignition tests then showed (percentages on dry weight of final sample),

14·0% stearic plus 8.2% oleic acids – Fired in 145 min;
Fired in 140 min.
14·0% palmitic plus 8.2% oleic acids – Fired in 151 min;
Fired in 152 min.

This reaction appears to be less violent than with oleic acid alone (see (10) above), so there has been no catalytic oxidation of the saturated acids by the oleic. Since the order of reaction is probably greater than zero with respect to fatty acid, the physical effect of dilution of oleic acid with saturated acid would be sufficient to explain this reduction in reactivity.

(13) Effect of Metal Catalysts

The fat soluble compounds of many metals are known to act as oxidation catalysts, and some are used for this purpose as driers in paints. It is usually accepted that only polyvalent metals can act in this way, the transition metals being particularly effective. Skellon (1950) has studied catalytic activity in relation to the Periodic Law, and has shown that different metals catalyse the reaction along different courses (e.g. peroxide formation, disappearance of double bonds, etc.). Skellon (1950) did not measure rates of heat generation, nor of oxygen absorption. Many authors have stated that cobalt is the most effective catalyst in promoting absorption of oxygen (e.g. Ellis, 1932; Briggs & Thomas, 1939). Both these investigators have shown that some metals are so effective as catalysts that they will promote oxidation of saturated acids such as stearic.

As Table 1 shows, iron and manganese are the only catalytic metals present in any significant amount in the pie wools analysed. To investigate the effects of metal catalysis, the technique described in (10) above was used to mix wool with fatty acids, and to these acids were added various quantities of catalysts in the form of metal resinates and stearates. The results of these experiments are given in Table 6. It can be seen that cobalt is an effective catalyst of oxidation of both oleic and stearic acids, and that iron also catalyses the oxidation of both acids. These experiments show that iron would catalyse the oxidation of oleic acid in pie fats; but in view of the relative stability of stearic acid, it is unlikely that iron catalysis of stearic acid would be of appreciable significance in the oxidation of pie wool.

(14) Oxidation of Natural Wool Wax

Walker and Williamson (1957) tested greasy wools sheared from various positions on living sheep, but found negligible heating. The possibilities were considered that wool wax might either catalyse or inhibit the demonstrated oxidation of oleic acid.

TABLE 6—Influence of Metal Catalysts on Oxidation of Oleic and Stearic Acids

Fatty Acid Added to Wool (on Dry Weight of Final Sample)	Metal Compound Added	Metal Content of Fat p.p.m.	Reaction With Oxygen at 100°C	
			Wool Temperature Rise °C	Time Taken to Reach Maximum Temperature (min)
4.5% oleic	Cobalt resinate	100	43	125
4.5% oleic	Cobalt resinate	300	37	125
4.5% oleic	Cobalt resinate	1000	Fired	50
4.5% oleic	Cobalt resinate	3000	Fired	27
4.5% oleic	Iron stearate	335	80	135
4.5% oleic	Iron stearate	1000	Fired	84
3.0% oleic plus 3.0% stearic	Iron stearate	1000	65	205
24.8% stearic	Cobalt resinate	100	Fired	263
24.7% stearic	Cobalt resinate	195	Fired	280
24.6% stearic	Cobalt resinate	390	Fired	333
23.6% stearic	Cobalt resinate	10,000	Fired	22
24.7% stearic	Iron stearate	2870	Fired	700

Wool wax was ether-extracted from wool sheared from cross-bred lambs. It was spread on wool as described in (*10*) above in various admixtures with oleic acid. Ignition tests then showed,

5.3% oleic acid plus 5.4% wool wax – Fired in 259 min.

10.1% oleic acid plus 5.0% wool wax – Fired in 115 min.

9.6% oleic acid plus 9.6% wool wax – Fired in 106 min.

As can be seen by comparison with (*10*) above, these figures provide no evidence of catalysis, and little evidence of strong inhibition. The reduction in reaction rate is again considered to be due mainly to the effect of dilution of a component contributing more than zero to the reaction order.

(*15*) EFFECT OF WOOL DIAMETER

Rapid oxidation of the fat in pie wool is made possible by the enormous surface area of wool. A typical head-piece pie wool has a mean fibre diameter of about 25 microns; and in calculations of area for present purposes, the fibre may be considered as a cylinder, since any local irregularities in the scales will be covered over by a film of fat. Such wool has an effective surface of 1.2×10^3 sq cm per g clean wool, and this will be slightly reduced by the presence of fat and the absence of equilibrium moisture in the present ignition experiments. This corresponds (at 20% of fat, say) to an area of about 5×10^3 sq cm per g fat, which may be compared with Ellis' (1950) figure of 10^3 for the dispersion of oleic and elaidic acids on filter paper, and Burgoyne and Thomas' (1951) figure of 2.5×10^4 for the dispersion of palm kernel fat on glass cloth.

To investigate whether variations in surface area might affect the reaction rate, clean wool "tops" were extracted with acetone in a Soxhlet, and mean

fibre diameter was measured by air flow (B.S. 3183:1959), conditioned to 50% RH. Two samples of "tops" were used having mean fibre diameters of 35·3 and 21·6 microns respectively, representing a ratio of 1·6 to 1 in surface area. Using the technique of (10) above, 8.0% of oleic acid (on the dry weight of the composite sample) was spread on each of these wools. This concentration was chosen to be a level at which the test would exercise a suitable degree of discrimination (see 10). Ignition tests on duplicate samples showed,

21.6 micron wool plus 8·0% oleic acid – Fired in 92 min;
                                         Fired in 83 min.
35·3 micron wool plus 8·0% oleic acid – Fired in 100 min;
                                         Fired in 85 min.

This variation in wool fibre diameter is seen to be without effect on the ignition test. This conclusion is confirmed by the experiments described in (10) above using various quantities of oleic acid spread on clean wool (29·4 microns diam.). These showed that rate of heat generation per unit weight of oleic acid is independent of the amount of oleic acid spread on the wool at surface areas between 4 and 12×10^3 sq cm per g oleic acid.

Some typical pie wools were also measured by the air flow method (after scouring), and the following mean diameters found at 50% RH,

Sample 446 – 29·3 microns

Sample 449 – 25·9 microns

Again the mean fibre diameter appears to be without significance.

DISCUSSION

Walker and Williamson (1957) ether-extracted fat from pie wools to demonstrate that the self-heating reaction is associated with the fat and not the wool. Confirmation of this view was provided by Carrie et al. (1959) who ether-extracted subcutaneous fat from freshly flayed sheep head pieces, and demonstrated that a sufficient quantity of this fat spread on clean wool would cause a fire in Walker and Williamson's test (1957). Walker and Williamson (1957) concluded that the heating reaction is caused by atmospheric oxidation of unsaturated components of the fat. The present experiments confirm this view by demonstrating absorption of oxygen, and by showing that the reaction has many features in common with the oxidation of other natural fats, and of unsaturated fatty acids; namely similar ratios of CO_2 evolved to O_2 absorbed, similar temperature coefficients of reaction, similar responses to oxygen partial pressure, and similar effects of inhibitors and catalysts. In addition, it has now been demonstrated that the behaviour of pie wool in Walker and Williamson's test (1957) can be duplicated by spreading sufficient quantities of oleic or elaidic acids (or their glycerides) on clean wool, again suggesting that the heating of pie wool is due to oxygen attack on these acids. The experiments with wool wax (together with those carried out by Walker and Williamson, 1957) show that in spite of the high iodine and peroxide values of this material (Walker and Williamson, 1957) it does not enter into the oxidation reaction at temperatures below say 120°C.

Walker and Williamson (1957) failed to discover any simple correlation between behaviour in their ignition test and analysis of pie wools. Many other investigators of the oxidation of oils and fats have also experienced this failure (e.g. Thompson, 1928; Garner, 1936; Kehren, 1939; Van Elteren, 1958). The present experiments do not fully clarify this situation, and demonstrate that the wide differences in behaviour between different pie wools are not explained by differences in polyethenoid content, in content or composition of fat not removed by ether, in cis-trans isomerism, nor in variations of mean fibre diameter. The wool wax which is present in variable amount in each pie fat may affect the situation, since it is relatively inert to oxidation at low temperatures. It is not likely that pie wool made by the hot tank process would retain much wool wax, although cold sweat pie wool might. The experiments with synthetic mixtures suggest that iron catalysis may be a major factor in controlling the reactivity of many pie wools.

Walker and Williamson (1957) describe five samples of pie wool which contained less than 7% of fat, and yet took fire in their test. Hilditch (1947) has stated that the fatty acid fraction of the external tissue fat of healthy sheep contains about 15% stearic, 28% palmitic, and 47% oleic acids. At the most, half of this 7% of pie fat could be oleic acid (olein and elaidic acid are even less reactive). The experiments of Table 6 show that catalytic iron could probably be responsible for a fire in the test at these fat concentrations. But other pie wools containing a little more fat took fire with a very small content of catalytic metals (e.g. 96, 219, 248, 305, 317, 438). A theory has been advanced by many authors (e.g. Garner, 1936) that organic catalysts of unknown composition exist in many fats; and it may be difficult to explain the reactivity of all pie wools without recourse to such a theory.

Fig. 1 illustrates the behaviour of various pie wools in Walker and Williamson's test (1957). Samples 449 and 233 are typical of a range of unscoured hot tank pie wools, relatively uncontaminated with iron, and containing about 15% of fat. As the figure shows, such wools can range from exhibiting considerable early heating (449) to very little (233). Samples 219 and 208 show practically no early heating, and probably represent a true induction period; such behaviour is rare in pie wool. Samples 44 and 2037 typify very reactive pie wools. Iron catalysis is well illustrated by 2037, 2007, and 905. Sample 2019 which reacted strongly initially and then cooled off probably also represents the effects of iron catalysis. This sample (2019) is particularly interesting since it also demonstrates the effects of order of reaction in a test of this kind. Several other wools showed less initial reaction, but ultimately took fire. Although the reactant atmosphere is replenished continuously, the reduction in concentration of reactive fat steadily diminishes the reaction rate. This effect would operate in spontaneous heating of baled wool in commerce also. Samples 22, 208, and 219 (Fig. 1) demonstrate that no indication of the final course of the test is given by the heating occurring during the first hour.

The significance of temperature coefficient of reaction in a spontaneous ignition mechanism is not simple. Most laboratory experiments on spon-

taneous ignition are carried out at elevated temperatures, a common temperature being the boiling point of water. A small temperature coefficient (i.e., small energy of activation, or large temperature interval for the rate to halve) means that any reaction demonstrated at 100°C will remain active at low temperatures. However, Frank-Kamenetskiĭ (1939) has derived an equation governing spontaneous ignition caused by a single reaction of zero order in piles of material with surface cooled to ambient temperature, which shows that the onset of thermal instability is directly proportional to reaction rate, and directly proportional to temperature coefficient (i.e. to the energy of activation, if the Arrhenius Equation is obeyed). Temperature coefficient and rate of reaction are thus of equal importance, and for spontaneous ignition to occur in any given pile of material a small temperature coefficient (small E) requires the reaction rate to be higher at the ambient temperature than does a large coefficient.

The temperature coefficients for samples 4 and 81 shown in Figs 2 and 3 make difficult a comparison in reactivity between the two wools. The ignition tests (Table 1) show that 81 is more reactive than 4, in pure oxygen at temperatures of 100°C and above. The oxygen absorption measurements (Figs. 2 & 3) demonstrate that this relationship is maintained down to 63°C. Since 81 is more influenced by change of temperature than is 4, a continued lowering of temperature must finally render 81 less reactive than 4 (provided the measured temperature coefficients continue to apply to sufficiently low temperatures). However, the difference in temperature coefficient between these two wools is not great, and in view of the similarity with other temperature coefficients quoted in the literature for oxidation of fats, it is doubtful if variations in temperature coefficient can be responsible for the sporadic occurrence of spontaneous pie wool fires in commerce. This is particularly true in view of the great differences in reactivity which can be demonstrated between wools under standardised conditions (see Table 1), and in view of the fact that every wool thus far found (by us) responsible for spontaneous ignition in commerce has fired in the test in say 40 minutes or less, e.g. samples 72, 81, 614, 2017. (c.f. Walker and Williamson, 1957).

Several authors have stated that there is a lower temperature limit, below which the influence of catalytic metals in the fat is not effective. Kehren (1939) suggests a minimum temperature of 85°C for catalysis of textile oleines by iron. Van Elteren (1958) has made an extensive investigation of this claim, and has shown that the catalytic influence of iron extends at least down to 35°C. There is thus no reason to think that a reaction under control of metal catalysts would have an anomalous temperature coefficient and so invalidate the above remarks.

As shown in Figs 4 and 5, samples 81 and 614 show marked differences in their response to oxygen partial pressure. Behaviour in oxygen partial pressures below those existing in air is particularly important, since absorption of oxygen in any heap or bale of reacting pie wool will reduce oxygen partial pressure in the centre. This is not as effective in preventing a temperature rise as might at first appear, since the outside layers of a sphere of material approaching a state of thermal instability (i.e. incipient spontaneous ignition) contribute more to temperature rise (and therefore

to the onset of thermal instability) than do the central layers (Chambré, 1952; Carrie, *et al.* 1959). However, experiments demonstrated that the reaction between pie wool and nitrogen (about 50 ppm oxygen) is almost negligible, and it seems likely from the present results that the rate of reaction of pie wool at oxygen partial pressures below 100 mm is almost directly proportional to oxygen partial pressure (i.e. a first order reaction with respect to oxygen). Any differences between pie wools in their response to oxygen partial pressure at these low oxygen pressures would be significant in influencing the course of spontaneous ignition under conditions of restricted access of air. No such differences are apparent between the two wools tested (81 and 614).

Burgoyne and Thomas (1951) distributed palm kernel fat over glass cloth, and exposed this to various oxygen pressures and studied the rate of reduction in pressure. They found this rate was much more dependent on oxygen pressure when it was below 50 mm than when it was higher, a result very similar to the present findings for pie wool. Burgoyne & Thomas (1951) concluded that at high oxygen pressures their reaction was controlled by chemical kinetics; but that at low oxygen pressures physical sorption of oxygen by the liquid fat became the rate-determining process. This last statement must mean that the reaction was limited by the rate of arrival of oxygen molecules at the interface, in fact by gaseous diffusion. Frank-Kamenetskiĭ (1955) has pointed out that since the gaseous diffusion coefficient varies with total pressure, all heterogeneous reactions taking place in a medium at rest and limited by gaseous diffusion will be zero order with respect to pressure of reactant gas, at constant gas composition. Burgoyne & Thomas (1951) carried out their experiments with nominally pure oxygen and allowed pressure to vary, and if their reaction had been limited by gaseous diffusion at low oxygen pressures it would have been zero order in this range and not first order. It is interesting to speculate what results Henderson & Young (1942) might have obtained had they worked at oxygen partial pressures lower than air. These authors worked at constant pressure and varied the composition of reactant gas, and if it be assumed that no absorption would have taken place in their experiments with no oxygen present, their reaction must have been approximately first order with respect to oxygen at compositions below that of air. The present results (using constant pressure) also suggest a reaction order (n) with respect to oxygen of $n \simeq 1$ at compositions below air, and n about $\frac{1}{2}$ at compositions above air. Frank-Kamenetskiĭ (1955) has shown that diffusion limited reactions are always first order with respect to gaseous reactant partial pressure under constant total pressure; but oxidations of unsaturated fats apparently show a change of order at about the oxygen partial pressure of air under conditions both of constant composition and of constant total pressure. It therefore seems that Burgoyne and Thomas' (1951) thesis that at low oxygen pressures the reaction is limited by physical sorption of oxygen, cannot be upheld. A full understanding of this phenomenon will probably have to await experiments in which reaction rate and oxygen partial pressure are varied independently at constant temperature, together with some means of overcoming the effects of reaction order with respect to the fat.

Acknowledgments

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THE OCCURRENCE IN NEW ZEALAND AND THE LIFE-HISTORY OF THE SOLDIER FLY *HERMETIA ILLUCENS* (L.) (DIPTERA: STRATIOMYIIDAE)

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Summary

The occurrence in New Zealand of *Hermetia illucens* (L.), one of the soldier flies, is recorded. Previous literature is reviewed and aspects of the biology and life-history of the fly are given. In the laboratory, eggs took 5 days in February and 7–14 days in April, at room temperature, to hatch. Larvae bred in a standard house-fly medium at 27·8°c pupated after 31 days. The pupation period was 9–10 days at 27·8°c and 15–17 days at room temperature during March. Larvae passed through six instars, the last of which showed major morphological changes. The size of mature larvae was found to be, on the average, smaller for males than for females. Overwintering occurred in the larval stage. The number of generations a year is considered to be no more than two, and possibly one only.

Introduction

In February 1956 a specimen of a stratiomyid fly was captured in Auckland. This was followed by another specimen in March of the same year, one in April 1957, and nine specimens during 1958 between February and April. Thereafter, numerous examples have been taken, mostly from areas near Auckland city, but including one from Pukekohe, 32 miles southwards.

Representative specimens were identified by Dr F. I. Van Emden of the Commonwealth Institute of Entomology, as *Hermetia illucens* (L.).

A live stratiomyid larva, of a size greater than any hitherto known from New Zealand, was taken from a compost heap in an Auckland garden during October 1956. This was allowed to pupate, the resulting imago being identified as *H. illucens*. Similar larvae from compost, in several Auckland localities, have since been reared to adults. It would thus appear that the fly is well established in the area.

The family Stratiomyiidae in New Zealand, as listed by Miller (1950), consists of 11 genera containing, in all, 29 species. Of these, *Altermetoponia rubriceps* (Macq.) a pasture pest localised in the Opotiki district, is a native of Australia, and *Neoexaireta spiniger* (Weid.) is of doubtful origin.

Hermetia illucens was described by Linnaeus in 1738. Copello (1926) recorded the insect as being active in Argentina from the middle of spring to the end of summer, laying its eggs on decomposing organic matter, or in the cracks in the front of bee hives. He stated that mating commenced in flight; that larvae hatch in 4–6 days, feed in the hive on pollen honey and those that survive the attacks of the bees pass through a pupal stage

of 2–20 days. If the bee colony is a weak one, these larvae may be able to destroy it. Buxton (Buxton and Hopkins, 1927) listed *H. illucens* among introduced insects in Samoa. He considered that the larvae were important in medical entomology as scavengers. They were bred from sour-sop fruit (*Anona*), from pumpkins and from dead crabs, all in an advanced state of decay. Borgmeier (1930) stated that the larvae live on all decaying plant and animal material from the rotting orange to the human corpse. James (1947) included the species amongst those causing myiasis in man in the United States of America. He described the larvae as breeding in decaying fruit and vegetables, catsup, animal cadavers, the wax, pollen, honey and waste materials found in beehives, and in outdoor privies. The large and vigorous larvae have been reported to cause rather severe gastro intestinal disturbances in several, apparently authentic, cases. He gave the world distribution of *H. illucens* which is summarised as follows: *Nearctic Region:* North America south of New York. *Neotropical Region:* Mexico, Central America, South America, West Indies. *Palaearctic Region:* Malta. *Australian Region:* Samoa, Guadalcanal, Bougainville, Hawaiian Islands.

Bohart and Gressitt (1951) found this stratiomyid present among the filth-inhabiting flies of Guam. They consider that because of its wide range of adult and larval food habits, there is a possibility of the adults carrying pathogens from excrement and corpses into houses. The species is included in keys to adults, eggs, larvae, and pupae.

There is evidence that *H. illucens* may be a significant factor in the natural control of house-flies (*Musca domestica* (L.)) in certain situations. In the U.S.A. Furman *et al.* (1959) have shown that where a dense population of stratiomyid larvae is present in poultry droppings, the more rapidly developing house-fly larvae appear to be starved out. It was discovered further by Kilpatrick and Schoof (1959) that when control of *M. domestica* in outdoor privies was attempted by the use of insecticides, the larvae of *H. illucens* were killed. Their absence created optimum breeding conditions which, coupled with house-fly resistance to some insecticides, allowed *M. domestica* to multiply rapidly.

An unusual breeding ground for *H. illucens* was recorded in 1959 from Hawaii. Large numbers of live and dead larvae, pupae and some dead adults were found in covered earthenware crocks containing tuna remains preserved in a solution of 10% formaldehyde. Some of the tuna had not completely submerged, but it was nevertheless moist and apparently impregnated with the preservative.

BIOLOGY AND LIFE-HISTORY

Adults

Hermetia illucens is readily distinguishable from all other stratiomyids in New Zealand by its much larger size (13–20 mm) and from all except *Neoexaireta spiniger* by its conspicuous black and white colouration. *N. spiniger* appears during the same months in the Auckland district and occupies a similar habitat. *H. illucens* may be separated from this species by the following characters which will also serve for its recognition:

HEAD: Black with some variable yellowish spots near the eyes. Vertex broad, bearing two flattened tubercles. ANTENNAE with apical segment spatulate. SCUTELLUM unarmed. ABDOMEN with second segment translucent creamy white, leaving, on the dorsal surface, lateral and median black lines which are narrow in the ♂, but wider and extending along the apical margin in the ♀, giving the white areas a triangular appearance. WINGS unpatterned, having a blue metallic sheen in life, but fading to dusky in dried specimens. LEGS with tarsi and basal third of hind tibiae conspicuous creamy white. The species is described in detail by Lindner (1938).

FIG. 1—Adult fly. Photo by S. A. Rumsey.

Adults are often noticed on windows of buildings during the summer months. All those so taken have been females, of which several were gravid, depositing eggs almost immediately, in the container in which they were placed. Reared adults have lived for approximately two weeks in captivity when moisture was made available to them.

Eggs

The eggs (Fig. 2a) are laid cemented together in a mass, as many as 1,062 having been counted from one ♀. They are elongate-oval, glabrous and creamy white in colour. Size: $1·4 \times 0·4$ mm. In the laboratory eggs took 5 days in February and 7–14 days in April to hatch at room temperature. Two hours before hatching (Fig. 2b) the chorion had become translucent and soft to the touch. The larva was clearly defined, segmentation,
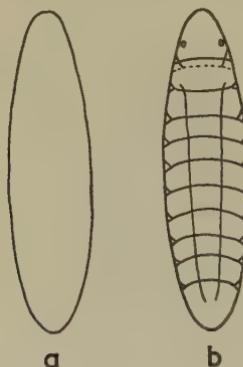
FIG. 2—a. Egg, 1 day after oviposition × 30. b. Egg, 2 hours before hatching. × 30.

main tracheal trunks and colouration of head and ocelli being visible. Emergence was completed in approximately 10 minutes, a hole being torn in the chorion by chitinous maxillary hooks and the larva emerging head first, propelled by a series of body movements assisted by the hooks. The chorion, now a transparent, papery skin, collapsed behind.

Larvae

Larvae reared in a constant temperature room (27·8°C), in semi-darkness, grew rapidly, some attaining a length of 17 mm after 12 days and their final instar after 21 days. The larval period, including the prepupal instar, occupied a minimum of 31 days. These larvae were bred in a standard housefly medium consisting of dried milk, yeast, water, and paper tissue.

Shortly before each moult occurred, the trachae and numerous bubbles became visible through the cuticle. The abdomen was distended several times, the head withdrawn from the old capsule, and the larva crawled out of the skin through a split which appeared laterally. This operation took less than one minute.

Larval progression is by undulating movements of the body, assisted by rows of stiff bristles and tooth-like projections on the ventral surface.

LARVAL INSTARS

The larvae were found to pass through six instars. This was determined by measurement of moulted head capsules checked with preserved, cultured specimens.

FIG. 3—Fifth instar and earlier stage larvae. Photo by S. A. Rumsey.

TABLE 1—Measurements in mm of Larval Head Capsules

Instar			Mean	Standard Deviation
1st	......	......	0·27–0·28 (2 spec. only)	—
2nd	......	......	0·45–0·46 (2 spec. only)	—
3rd	......	......	0·68	0·09
4th	......	......	1·26	0·09
5th	......	......	2·02	0·36
6th	......	......	2·37	0·29

First to Fourth Instars

The newly hatched larva is opaque, creamy white, with head castaneous. Size: 1·8 × 0.4 mm. Colouration remains the same up to and including the fourth instar. The only noticeable difference, apart from size, being the slightly altered outlines of the head.

Fifth Instar

In the laboratory, the first fifth instar larva was noticed 11 days after the eggs were hatched. It is in this stage that full size is attained. A day or two after moulting, the cuticle takes on a shagreened appearance and darkens to greyish yellow.
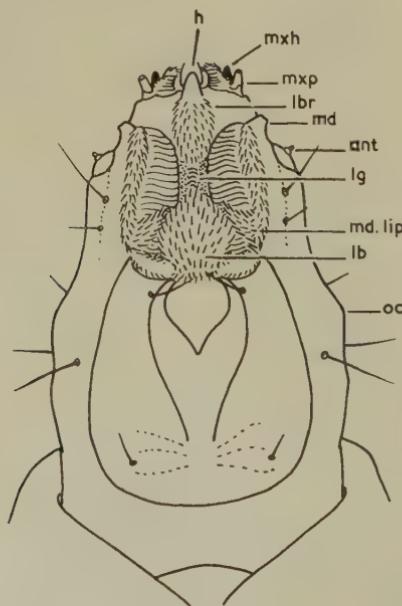
FIG. 4—Fifth instar, head, ventral view. × 32.

The mouthparts were described by Borgmeier (1930). The labrum, ligula and labium are all membranous and finely pubescent. The maxillae, also pubescent, bear a strongly chitinised two-hooked process. The palps are tubular. The mandibles, which are fused to the genae, bear finely pubescent lips extended into a curved finger-like process.

Sixth Instar

The sixth instar, which is also the prepupal stage, first appeared 18 days after hatching. After moulting, the cuticle became very dark and the pubescence longer and coarser.
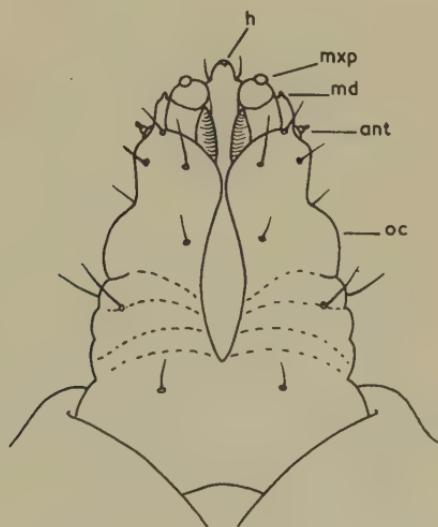
Fig. 5—Sixth instar, head, ventral view. × 32.

Abbreviations used in figures 4 and 5. ant., antenna; h., horn; lb., labium; lbr., labrum; lg., ligula; md., mandible; md. lip., lip of mandible; mxh., maxillary hook; mxp., maxillary palp; oc., ocellus.

In this stage the head is more strongly chitinised and the ocelli have become prominent. The mouthparts are reduced and immobile. When some were deprived of moisture for long periods they continued to live, and it was therefore considered that the sixth instar larvae did not feed. However, after these had been left in coloured fly medium for three days, the gut, on dissection, was found to be similarly coloured, showing that at least some part of the substance had been ingested.

The small, black, flattened, tooth-like processes, visible ventrally on the abdominal segments of younger larvae, are lacking in the sixth instar and there are some additional setae. The mature larva is described and figured by Petersen (1951). Head, mouthparts, chaetotaxy and the manner in which these differ from those of the fifth instar, are described and figured by Borgmeier (1930). The larva figured by Ricardo (1929) is fifth instar.
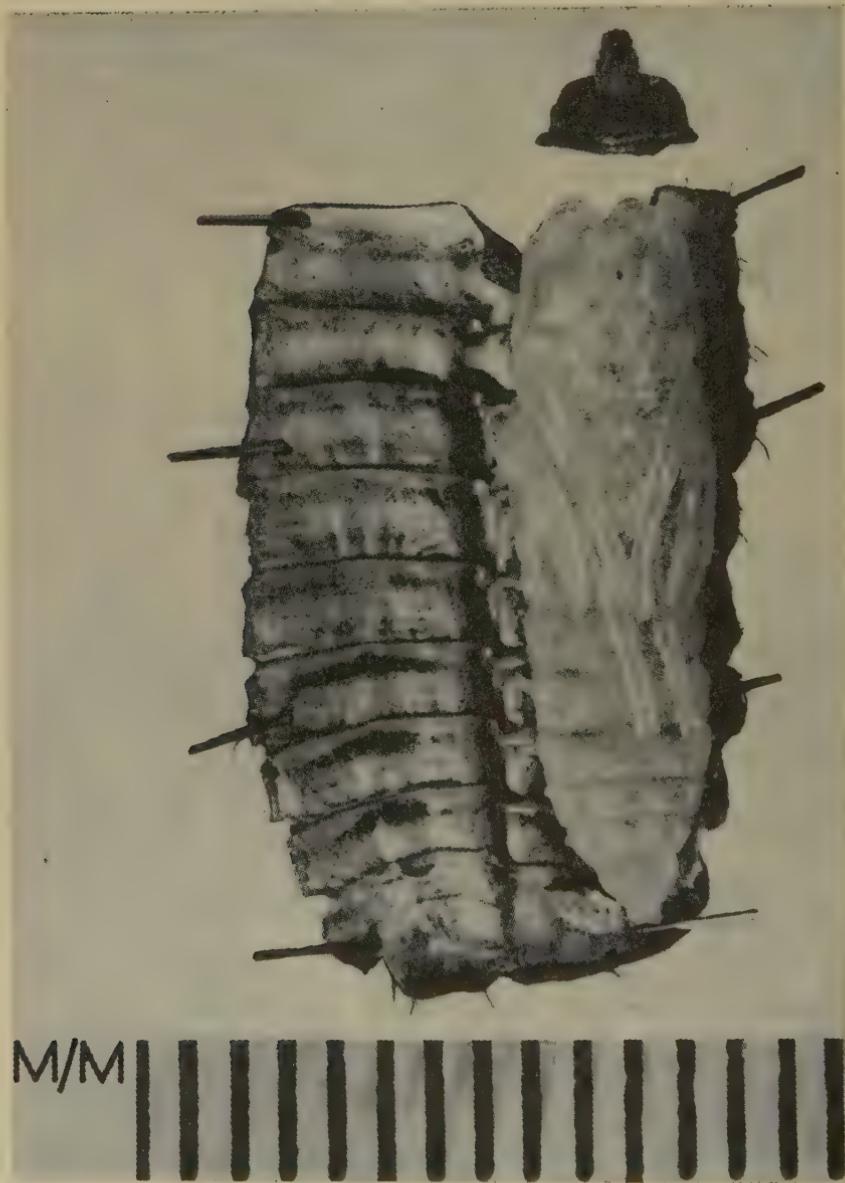
Fig. 6—Pupa (dissected). Photo by S. A. Rumsey.

Pupae

As with other stratiomyids, pupation takes place within the larval skin (Fig. 6). Before this occurred, the prepupae arranged themselves vertically in the medium with heads protruding above the surface. The cuticle became

M/M

a   b

Fig. 7—a. Pupa, ventral view. b. Pupal exuvia, ventral view. Photo by S. A. Rumsey.

rigid with the two posterior segments curved ventrally (Fig. 7a). To effect emergence, the head capsule together with the first thoracic segment was pushed off. A mid-dorsal split appeared in the second and third segments, and the mature imago struggled through the resulting hole (Fig. 7b).

The pupation period was 9–10 days in the constant temperature room, and 15–17 days at room temperature during March. The first adult emerged 50 days from oviposition.

Sex could not be determined with certainty from the size of the mature larva, but the following evidence shows that, on the average, the males are smaller than the females. Prepupae measuring 17–18 mm were separated from those 21–23 mm. Both sexes emerged from each group but males predominated in the 17–18 mm group. (21♂♂, 11♀♀) and females in the 21–23 mm group (51♀♀, 22♂♂). Larvae from compost have measured up to 26 × 6 mm.

Overwintering

In the field, adults have been taken from mid-November to late May. Although three generations a year would appear to be possible under optimum conditions, there are probably no more than two and possibly only a single generation, as is stated to be the case in Argentina (Copello, 1926).

The insect overwinters in the larval stage, low temperatures reducing it to a state of quiescence. Specimens collected in the field in June were decidedly sluggish compared with those collected in March or with cultured specimens. Growth at once recommenced when warmth and food were made available. A few larvae collected outdoors on 27 May 1959 were left unattended in a jar of soil until 8 March 1960. One was still alive, possibly having fed on the remains of the others. This one was placed in some culture medium in the constant temperature room. It fed and grew rapidly, moulted on 29 March to the final instar, pupated 23–25 April and emerged as an adult on 17 May, almost a year from its capture.

The following specimens are held in the collection of Plant Diseases Division, D.S.I.R.

Adults

Auckland: 21.III.1956, 1♀, (G. Cronin); Mt Albert, — II, 1956, 1♀, (G. Chamberlain); 19.III.1958, 1♀; 23.III.1958, 1♀; 24.III.1958, 1♀; 31.I.1960, 1♀; in house, 9.III.1959, 1♀; 24.III.1959, 1♀; on window, 15.IV.1959, 1♀, (B. M. May); —.III.1959, 1♀; on window, 28.III.1959, 1♀; 28.II.1960, 1♀, (R. A. Harrison); in house, 25.IV.1960, 1♀, (M. L. Harrison); Epsom, larva ex compost heap, 17.X.1956, 1♀, (H. J. Hartfield); 23.III.1958, 1♀; inside house, 14.IV.1957, 1♀, (D. Spiller); ex compost, 11.III.1960, 1♂, (R. A. Harrison); Owairaka, Plant Diseases Division, 26.III.1959, 1♀; ex lab., 19.II.1958, 1♀; 3.IV.1958, 1♀, (R. A. Harrison); ex lab., 28.III.1958, 1♀, (K. P. Lamb); ex lab., 7.II.1958, 1♀, (A. M. Richards); on window, 3.IV.1958, 1♀, (H. Jafar); on window, 14.IV.1959, 1♀, (K. A. J. Wise); ex office, 25.I.1960, 1♀, (D. Currie); ex insectary, 18.III.1960, 1♀; ex compost, 27.V.1959, 1♀, pupated 23–25.IV.1960, emerged 17.V.1960, (B. M. May); Kingsland, on window, 24.V.1959, 1♀, (B. E. May); Mangere, 4.IV.1960, 1♂, (K. A. J. Wise); Avondale, in garden, 21.XII.1959, 1♀; 14.II.1960, 1♀; on tomato leaf near compost heap, 16.XII.1959, 1♂, (D. J. McCorkindale) Pukekohe: ex garden, 14.IV.1959, 1♀, (Dept. Agric.).

Larvae

Auckland: Owairaka, Plant Diseases Division, 21.IV.1959, 1 newly emerged 1st instar and empty chorion of egg laid 14.IV.1959; 24.IV.1959,

5 1st. ins. from eggs laid 14–15.IV.1959; *ex* rotting tomato haulms, 2.VI.1959, 17 ex.; dead *ex* jar in lab., 20.VII.1959, 3 ex.; 15.IX.1959, 4 ex.; *ex* culture of eggs hatched 6.II.1960, 11.II.1960, 1 2nd ins., 1 3rd ins.; 15.II.1960, 6 4th ins.; 16.II.1960, 4 4th ins.; 1 5th ins.; 18.II.1960, 6 5th ins.; 21.II.1960, 4 5th ins.; 24.II.1960, 1 4th ins.; 5 5th ins.; 1 6th ins. with 5th ins. exuvia still attached; 26.II.1960, 5 5th ins., 2 6th ins.; 29.II.1960, 4 5th ins., 3 6th ins.; 4.III.1960, 1 5th ins., 3 6th ins.; 7.II.1960, 1 5th ins., 7 6th ins.; 5–6.III.1960, 2 pupae (dissected); 21.III.1960, 5 pupae, (*B. M. May*); Avondale, *ex* garden soil mixed with compost, 11.I.1960, 1 6th ins.; *ex* compost, 3.III.1960, 1 6th ins., 1 pupa (dissected), (*D. J. McCorkindale*); Epsom, *ex* compost, 11.II.1960, 2 6th ins., (*R. A. Harrison*).

EGGS

AUCKLAND: Avondale, 14.II.1960, 1062 laid by 1♀, (*D. J. McCorkindale*).

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A GEL FRACTION OF WHEAT GLUTEN; MIXING, OXIDATION, AND LIPID RELATIONSHIPS

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Summary

Part of the protein-lipid complex of wheat gluten has a gelatinous character, is insoluble in dilute aqueous formic acid and may be rendered soluble by oxidation. Variation in the proportion of this gel fraction has been explored as a measure of the degree of structure within dough, and the effects of mixing and of treatment with bromate and iodate have been examined in this way. Four types of mixing gave similar patterns of structure building and breakdown. The extent of the bromate reaction is dependent primarily on total reaction time whilst the extent of the iodate reaction is dependent primarily on mixing time. Two types of binding of lipid to protein during doughing have been demonstrated. The interconvertibility of some acid-soluble and acid-insoluble protein fractions of dough has been shown.

Introduction

Recent study of wheat gluten proteins by partial solubility methods has shown that fractionation of the complex may be achieved with dilute formic acid provided mechanical treatment such as would break the gel structure is avoided (Meredith, Sammons and Frazer, 1960 b). The acid-insoluble gel protein may be the structural unit of gluten.

Determination of the proportion of gel present in gluten should thus provide an analytical tool for investigating many of the changes which a dough may undergo. Studies have been made of the effects of work on dough and of the effects of oxidation by bromate and iodate. This analysis for gel structure is perhaps similar to the determinations of glutenin made by many earlier investigators, such as Grewe and Bailey (1927) but, since many variables have been taken into account, it may be considered more precise. That is not to claim, however, that a precise analytical method has been developed.

From the present work it appears that the structural gel is in equilibrium with a part of the acid-soluble protein, and the reversibility of this labile system is demonstrated. The gel also binds lipid of the flour into the gluten complex by several mechanisms.

Materials and Methods

Most of the work was carried out with normal bakers' flours milled to 78% extraction from New Zealand wheats with no additions or treatments. The work on the lipid-binding properties was carried out with an English bakers' flour having creta and vitamins added but no treatment applied.

For the assay of gel structure, dough was prepared with a solution containing 1% sodium chloride and 100 p.p.m versene, in the proportion of 2 parts of solution to 3 parts of flour, by weight. After appropriate working the dough was rested under solution for about 1 hr. Gluten was then washed from the mass with a stream of tap water at about 25°C, over a 140 mesh wire sieve when necessary, rested under water for 1 hr, drained without additional work being performed on it, and freeze-dried. The dry gluten was roughly ground in a mortar.

500 ± 5 mg powdered dry gluten was weighed into a 50 ml conical centrifuge tube and 15 ml of 0·01N formic acid (containing 50 p.p.m. versene) added. The contents of the stoppered tube were mixed by gentle inversion six times. This mixing procedure was repeated on three further occasions, at 10 min. intervals, and the suspensions were left to stand 1½ hr. After centrifuging, the supernatant solution (acid-soluble protein) was decanted into a tared beaker. A further 10 ml of dilute acid was added to the residue in the tube and the contents mixed twice as before. The tube was centrifuged after 1 hr and the supernatant solution decanted into the same beaker.

To the remaining gel was added 1 ml N formic acid solution and 1 ml 30% hydrogen peroxide solution; the contents of the tube were mixed by vigorous shaking and incubated at 30°C for at least 5 hr for oxidation of the gel to occur. The mixture was centrifuged and the turbid supernatant (gel protein) decanted into a second tared beaker. The residue of starch and traces of bran and gum was discarded. The two protein solutions were evaporated and dried at 110°C overnight, cooled in a desiccator and weighed. The gel protein is expressed as percentage of acid-soluble plus gel protein.

In the mixing experiments, the Farinograph used had a bronze bowl taking 300 g flour and driven at 60 r.p.m. The pin mixer was of the McDuffee type, modified from a Hobart food mixer. The plunger mixer comprised a 18 mm diameter plunger moving up and down 100 times a minute and placed eccentrically in a 33 mm diameter cylinder which rotated at 12 r.p.m.; a piston with a hole for the plunger to pass through lay on the surface of the dough and kept it in the cylinder.

"No dough-work" glutens were prepared by sprinkling 25 g flour on to the surface of 500 ml of saline-versene solution in a shallow dish. After 2 hours the sludge was poured on to a 140 mesh wire sieve, washed with tap water, and the accumulated gluten worked in the fingers to wash out excess starch. The residue of gummy material and bran left on the sieve was discarded.

Extraction of lipid from flour was by percolation at room temperature, first with dry ether, then with ethanol-ether (3 : 1) mixture. Chromatographic analysis of lipid mixtures was carried out on silica-celite columns (Fillerup and Mead 1953) (Mead and Fillerup 1954). Fraction 1, containing hydrocarbons, was eluted with petroleum ether only; fraction 2, containing sterol esters, with 1% ether in petroleum; fraction 3, containing a mixture of glycerides, with 3% ether in petroleum; fraction 4, containing free sterol and glycerides, with 10% ether in petroleum; fraction 5,

containing free fatty acids and other material, with 50% ether in petroleum; fraction 6, containing phospholipids and other complex lipids, with absolute methanol.

Lipid-free protein fractions A, F, and E for the lipid-binding studies were prepared as described previously (Meredith, Sammons and Frazer 1960 a, b). Fraction A is the part of the gluten protein that is soluble in aqueous acid and in methanol-chloroform (1 : 2) mixture; fraction F is soluble in acid but insoluble in methanol-chloroform; fraction E (gel) is insoluble in both solvent systems.

Experimental

The Actions of Potassium Bromate and Iodate on Dough

The effects of varying concentrations of bromide, bromate and iodate in the presence of versene on doughs made with saline solution, mixed by hand for 25 minutes and rested for 6 hours at 30°c are shown in Fig. 1.
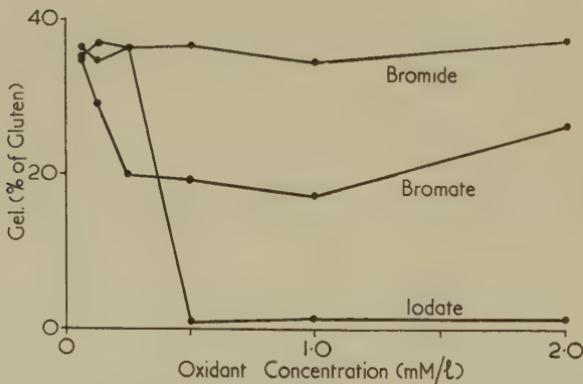
Fig. 1—Effect of concentration of potassium bromide, bromate, and iodate in dough liquor on the proportion of gel in gluten.

From these curves concentrations of bromate and iodate were chosen (1·0 and 0·4 mM/l respectively) for an investigation of the effects of time of working of the dough and of total reaction time, at constant concentration and temperature. The results are presented in Fig. 2 and it is evident that the action of bromate on the protein is highly dependent on the total reaction time and that increase in mixing time annuls the action. On the other hand, the action of iodate is almost independent of the total reaction time but depends directly on the mixing time.

The Effect of Mechanical Work on Dough, Without Added Oxidant

Work done on a dough may be of diverse kinds, *viz.* compression, extension, sheer, torsion, bending, and the three machines investigated have actions which differ widely and all are quite different from hand
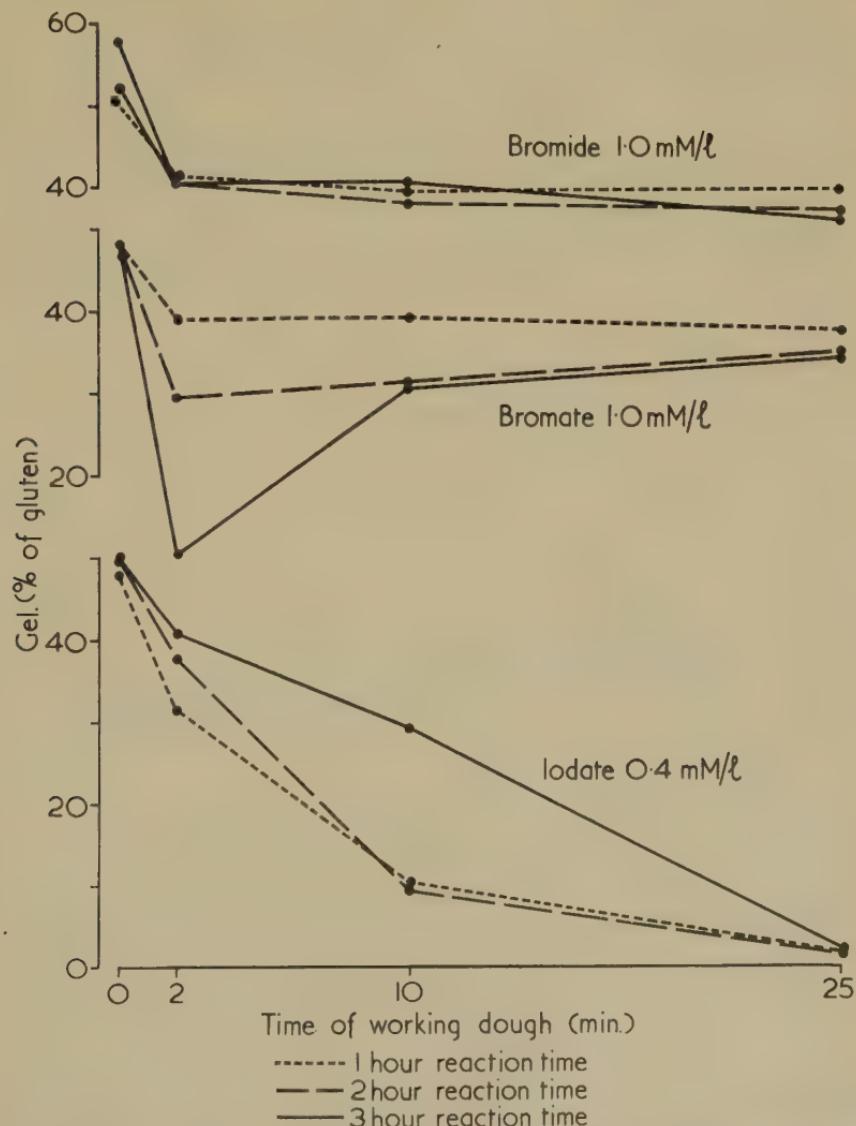
FIG. 2—Effects of time of working dough and total reaction time on the proportion of gel in gluten. Doughs treated with potassium bromide, bromate, and iodate.

working. The amount of work performed on the dough could not be determined except for the Farinograph, so gel percentages are expressed in Fig. 3 relative to the time of mixing, together with the corresponding Farinogram. All the results are for one flour with saline-versene solution at 67% absorption, which gave a maximum consistency of 460 Brabender units after 4½ minutes mixing in the Farinograph. Each experiment was carried out on two occasions,
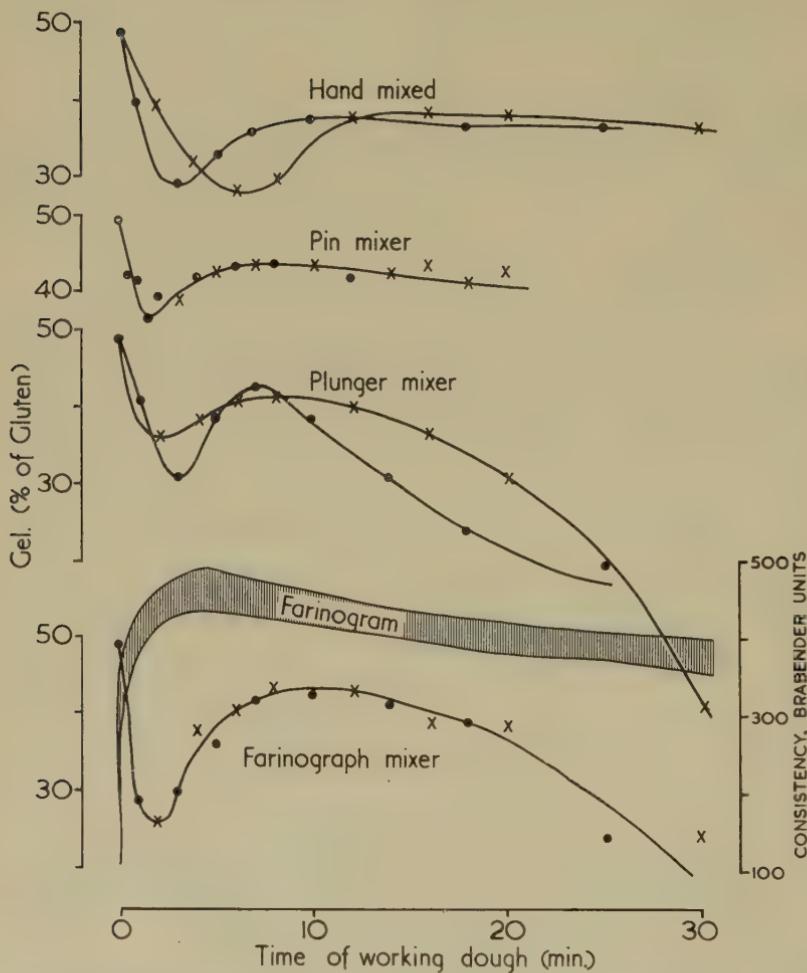
Fig. 3—Effects of time and method of mixing dough on the proportion of gel in gluten. The farinogram of the dough is also shown.

After 8 to 10 minutes in the pin mixer, the dough spread uniformly around the bowl and from this point the mixer failed to work the dough. Doughs for test baking are normally taken from the pin mixer at 1½ minutes, which corresponds to the minimum of the gel curve. The zero time point is identical for all the curves and is derived from the "no dough-work" experiment.

The curves are all of the same general shape, though their time scales differ. Those for the three machines have maxima of about 42%–43% gel whereas hand mixing produced only 38% gel at the maximum. The

minima can be assessed reasonably with respect to time but not with respect
to gel content since the curves are steep at this point. Neither the mini-
mum, at 2 minutes, nor the maximum, at 10 minutes, of the gel curve of
the Farinograph mixer corresponds to the maximum of the Farinogram at
$4\frac{1}{2}$ minutes.

The yield of gluten from doughs mixed for varying periods in the
Farinograph was determined and expressed as nitrogen recovered in the
gluten. It can be seen from Table 1 that the recovery was not complete
until $1\frac{1}{2}$ minutes of mixing had taken place. The work involved in washing
the dough piece probably reduces considerably the magnitude of the effect.

TABLE 1—Yield of Gluten from Dough Mixed in Farinograph

Mixing Time (min)	Gluten N / Flour N (%)
0	54.5
$\frac{1}{2}$	83.4
1	85.0
$1\frac{1}{2}$	87.3
2	86.7
3	86.7
5	86.2
10	86.2
20	86.7

The Lipid Binding Properties of the Gel Protein

Hand washed gluten, extracted with chloroform after evaporation with
ethanol to dissociate protein – lipid complexes, yielded 59% of the lipid of
the flour from which the gluten had been prepared. On fractionating such
gluten with dilute formic acid 88% of its lipid was found associated with
the gel fraction (which comprised about 40% of the protein). Thus in
gluten the gel fraction is the main lipid-binding agent.

Experimental systems were set up in which about 100 mg of total flour
lipid was mixed with 500 mg of a lipid-free protein fraction in petroleum
suspension, the mixture evaporated to dryness at low temperature and then
hydrated with dilute aqueous formic acid solution. Free lipid was removed
from the mixture by repeated extractions with petroleum. In this way it was
found that fraction E bound 46% of the lipid, fraction F bound 35% and
fraction A bound only 16%. Thus fractions E and F have greater lipid
binding power than has fraction A. Similar experiments showed that the
gel protein (fraction E) bound 39% of the ether-extracted flour lipid and
74% of the ethanol-extracted lipid. The calculated mean for the whole flour
lipid is 48% bound. It thus appears that in the binding mechanism there
is some preference for those lipids not extracted from flour by ether
alone.

In the experiment involving gel protein and ethanol-extracted lipid, the original lipid contained 1·41% phosphorus whereas the lipid that had not combined with the gel contained 0·90% phosphorus. There had thus been preferential binding of phosphorus-containing lipid.

Lipid analyses from an experiment in which total flour lipid was reacted with gel protein are given in Table 2 and from this it seems that the binding has been relatively non-specific with respect to the total lipids, although there has been some preference for the complex lipid fraction.

TABLE 2—Composition of Lipid Mixtures

Fraction	Total Extract of Flour		Ether Extract of Flour	
	A*	B*	A	B
1 Hydrocarbons	1.2	3.1	0.5	2.0
2 Sterol esters	4.8	5.0	5.5	4.4
3 Glycerides			36.3	35.6
4 Sterols & Glycerides	36.9	46.2	8.1	8.0
5 Fatty acids			12.0	13.5
6 Complex lipids	57.1	45.7	37.6	36.5

*A shows composition of the original lipid mixture %.   B shows composition of that part of the lipid not bound by the gel protein. %.

A similar experiment (Table 2) showed no preferential binding with respect to the ether-extractable lipids of flour.

These lipid studies thus show that the gel protein fraction has a high affinity for all the lipid fractions of flour and a rather higher affinity for a phosphorus-containing complex lipid fraction not extracted from flour by ether.

The Equilibrium Nature of the Gel Fraction

It has been shown (Meredith, Sammons & Frazer, 1960) that the gel protein may pass to an acid-soluble form through mechanical breakdown, oxidation or leaching with acid until the structure expands beyond stability. Furthermore, experiments already described in this paper show that a variable proportion of gel protein is found in gluten from the same flour, depending on the prior treatment of the dough. It is evident, then, that a reversible system of gel protein and acid-soluble protein exists in gluten. Two further observations show that the reverse change is possible.

Gliadin was extracted with 55% ethanol from flour which had first been extracted with 10% sodium chloride solution. It was partitioned with dilute formic acid, yielding 12% gel fraction and 88% acid-soluble fraction. The acid-soluble fraction was partitioned with methanol-chloroform (1 : 2) mixture, giving soluble (A) and insoluble (F) fractions which were

50% and 38% respectively of the original gliadin. On assay for gel content by the procedure used for doughs, the acid-soluble gliadin contained 0·7% gel, fraction A contained 0·9% and fraction F contained 68·5% gel. It seemed possible that the results could be explained by fraction A protein carrying the normally acid-insoluble protein into solution, along the lines suggested by Kuhlmann (1937). This explanation has to be rejected since a mixture of equal weights of fractions A and F assayed 31·3% gel, conclusively demonstrating that gel protein was not being carried into solution by the soluble protein to any appreciable extent. Thus we must conclude that a change has occurred in the protein of fraction F from an acid-soluble to a largely acid-insoluble form as a result of the treatment with methanol-chloroform mixture.

Variation was found within the samples of powdered gluten on keeping them for a few days before analysis for gel content. As example, two curves of gel protein proportion against mixing time are shown in Fig. 4. Analyses were carried out on the same dried glutens after one day and after storage for a further seven days at room temperature, in the dark, in air. It is obvious from these and similar observations that considerable changes can occur on standing in the air-dry state.
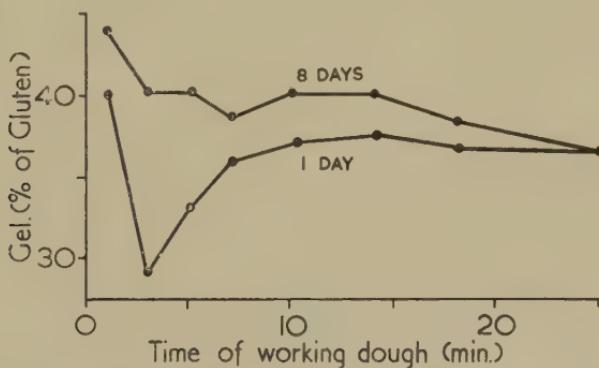
FIG. 4—Effect of duration of storage of air-dry gluten on the proportion of gel found.

DISCUSSION

The nature of the fractionation will be discussed before considering the results obtained by the method. As applied in the assay method here presented, the fractionation is highly arbitrary. The gels which result from the acid leaching of gluten differ in a qualitative as well as in a quantitative sense, and interesting results might have been obtained from measurements of gel strength. This qualitative difference in gels has been partly allowed for by using a double extraction of the gel with acid. During the second extraction, when the cation content of the material is low, the weaker gel structures are in some degree dispersed. It is then only the more rigid structure which has been determined as 'gel protein' and there is more contrast

in the experimental observations than might have been seen had only a single extraction been made. The experimental variation of the results, as seen in the bromide curve of Fig. 1, is larger than could be desired, but this was minimised by inclusion of sodium chloride and versene in the doughing medium. 1% sodium chloride makes the total ionic strength of the system so high that variations in flour ash or added oxidant are negligible. At low ionic strength the physical state of the gel protein is highly sensitive to changes in cation concentration, whereas at high ionic strength it is relatively insensitive to such changes. The addition of versene not only reduced experimental variation but, in agreement with the work of Hlynka (1957), increased the extent of reaction with low concentrations of bromate.

The fractionation as originally described was carried out on purified glutens prepared from lipid-free flours. Additional work has shown that for use merely as an analytical tool in studying reaction mechanisms such purified preparations are unnecessary. In the assay method crude glutens containing starch, bran, pentosans, and lipids besides the protein are used. By oxidising the gel to the soluble form, those materials such as starch grains, bran particles, and pentosan gum which are not solubilised by the oxidation are excluded from the analysis. The lipid is partitioned by the acid treatment between the soluble and insoluble protein fractions. The major part remains with the gel protein and after oxidation is emulsified in the protein solution. Although it is conceivable that the partitioning of the lipid between the two protein fractions differs after partial oxidation, such changes have been ignored in the present investigations and only the overall fractionation of protein plus lipid has been studied. The experiments with oxidising agents showed that the extent of the action of bromate on the protein structure depended on the duration of reaction after mixing, whilst increased mixing time reversed the effect. The action of iodate depended little on reaction time but was governed largely by the duration of mixing. These experiments thus reinforce the view already held that bromate and iodate react in dough by different mechanisms. Iodate reacts immediately and drastically whilst bromate reacts slowly. Bloksma and Hlynka (1960) have shown that remixing a dough opposes the effect of excess bromate, especially with increase in reaction time, as judged by relaxation curves. A way has been pointed for further experimental work to elucidate the reaction mechanisms involved.

The mixing studies are possibly the most important piece of work presented since they showed the similarity of the structure building and breaking reaction sequences in dough whatever mixing method was used. There was initially a fall in structural content, followed closely by a rise. Later there was a more marked and prolonged fall in structural content if working had been drastic enough.

Hess (1953, 1954) has shown that the endosperm fragments of wheat flour contain two separate protein fractions which he terms 'fixed' and 'wedge' protein. In doughing the protein fractions and the starch are brought into intimate contact. Of the adherence of the protein to the starch granules and the formation of structure within the protein, the latter is

obviously the more important consideration since on washing dough in water most of the protein remains as the coherent gluten mass whilst the starch washes free. Thus during doughing the 'fixed' protein has dissociated itself from the starch granule and associated with the 'wedge' protein. Similar ideas of dough structure and the effect of mixing were presented by Callow in 1938.

To explain the curve of mixing time versus gel protein it is postulated that the initial part of the mixing curve represents the freeing of the 'fixed' protein from the starch granules, so that in the early stages the protein found in the gluten is mostly derived from the 'wedge' protein. This is reflected also in the amount of gluten which is found, although the magnitude of such differences as are found is greatly reduced by the work the dough piece received in washing out the gluten. The minimum of the curve then corresponds to the condition where the 'fixed' protein has transferred from the starch grains to the 'wedge' protein but has not yet been bound into the gel structure. The full complement of protein is thus found in the gluten but the structure found is due mainly to the pre-existing component. The rising part of the curve must show the formation of structure within the protein combination, until a point is reached where the rate of structural formation is exceeded by the rate of mechanical disintegration, which may involve the formation of more stable, but less easily formed, bonds. We may presume that structure building involves intermolecular bonding whilst breakdown involves intramolecular bond formation.

It is, of course, not the protein alone that is involved in this structure building and breakdown and Grosskreutz (1960) has shown that the presence of lipid in gluten is required for full structural formation. Traub, Hutchinson and Daniels (1957) have similarly shown that lipid plays a part in gluten structure. That lipid of flour becomes bound to the gluten protein in doughing, or even on wetting flour, was shown by Olcott and Mecham (1947) and by earlier workers. The work of Bloksma (1959) on recovery of gluten with varying time of mixing in the Farinograph is in accord with the present findings and is consistent with the idea that the presence of native lipid facilitates the transfer of protein from starch to protein-complex. The experiments here described on the binding of lipids confirm and extend the earlier work. There is evidently more than one binding mechanism possible, since both a non-specific general binding of all types of lipid present and a specific preference for the complex lipids was found. The lipids of flour are indeed complex in structure as shown by the recent studies of Daniels (1958) and Carter *et al.* (1956) (1958). The non-specific binding of lipid to the gel protein and the formation of the protein complex of gluten may have something in common, since the most soluble fraction of the gluten proteins (fraction A) is almost non-ionic and has considerable lipid character (Meredith, Sammons & Frazer 1960 a). Lipid is not an essential intermediate in binding the protein fractions since gluten containing most of the protein of flour may be prepared just as readily from lipid-free as from lipid-containing flour. Although part of the lipid of flour is derived from the germ (Stevens 1959), a considerable part is derived from the endosperm itself and it is interesting to

speculate why the lipid is not already bound to the protein in the endo-sperm of the wheat grain.

That an equilibrium exists between the structural protein and part of the soluble protein of gluten is a basic theme of this discussion. Evidence has been produced that gross changes from soluble to structural forms and the reverse are possible under extreme conditions and that smaller changes occur under the milder conditions of air-dry storage and of oxidation at the concentrations used for improvement in baking. Schaeffer *et al.* (1960) have shown that change from acid-soluble to acid-insoluble form occurs when lyophilized gluten is wetted with aqueous alcohol and when gluten is heated in water.

It was hoped that determination of the relative amounts of protein frac-tions might lead to some analytical assessment of flour quality and two series of experiments were carried out to look for a relation between the pro-portion or quantity of gel protein in dough and the protein quality judged by baking performance. The findings were inconclusive and the experi-ments were undoubtedly premature since the effects of mixing time and of oxidation were not then understood. It is probable that other variables must also be controlled before such a relation could be found. Direct determina-tion of the amount of gel protein in flour is allied to the sedimentation test for wheat quality (Pinckney, Greenaway & Zeleny, 1957), though the effects of starch and bran have been removed and the amount of protein is divorced from its rate of settling.

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THE ANTICHOLINESTERASE ACTIVITY OF DEMETON-METHYL

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Summary

A study has been made of the *in vivo* anticholinesterase activity of demeton-methyl following oral administration to rabbits and of the *in vitro* activity using rabbit serum and human plasma. *In vitro* human plasma anticholinesterase activities of demeton-S-methyl, demeton-O-methyl, the sulphoxide, and the sulphone of demeton-methyl, demeton, malathion and Rogor were also measured for comparative purposes.

Demeton-S-methyl is a stronger *in vitro* inhibitor than demeton-O-methyl, the sulphoxide or the sulphone, but only one quarter as strong as demeton.

The *in vitro* rabbit serum anticholinesterase activity of demeton-S-methyl was 22 times the oral *in vivo* activity in rabbits, thus suggesting that demeton-methyl is extensively inactivated in the digestive system of the rabbit.

A modified Michel (1949) method has been described which provides improved accuracy and sensitivity in determination of cholinesterase levels.

Introduction

Demeton-methyl, known also as Metasystox, is the common name for the mixed isomers, OO dimethyl (O) ethylthioethyl phosphorothionate (demeton-O-methyl) and OO dimethyl (S) ethylthioethyl phosphorothiolate (demeton-S-methyl). The more toxic product, demeton-S-methyl, is claimed by the manufacturers, Farbenfabriken Bayer AG (1955) to have an oral LD_{50} (rat) of 40 mg/kg, while the LD_{50} of demeton-O-methyl is 180 mg/kg. The isomer mixture demeton-S-methyl : demeton-O-methyl is present in the commercial preparation in a ratio of 30 : 70. The LD_{50} (rat) of the mixture is claimed to be 120 mg/kg.

Demeton-methyl is a systemic insecticide and has found application in New Zealand in control of aphids on brassicas and on apples.

The manufacturers of demeton methyl, Farbenfabriken Bayer AG, have carried out toxicity and residue studies on radioactive ^{32}P-labelled demeton-methyl and have shown that demeton-S-methyl is converted to the sulphoxide in the plant and can be further oxidised to the sulphone. Both the metabolites exhibit some toxicity (Heath and Vandekar 1957). Demeton-methyl is claimed to be a weak inhibitor of cholinesterase, but Heath and Vandekar have shown that under certain conditions a strongly inhibiting sulphonium derivative can be formed.

The present work was initiated as part of an investigation into the residues remaining in treated crops and, in view of the foregoing, it was deemed advisable to determine these residues by measuring the anti-cholinesterase activity. Several methods have been published for determining the cholinesterase levels in blood (Michel (1949), Aldridge (1950), and Metcalf (1951)). The present work initially utilised an *in vitro* cholinesterase inhibition method based on the work of Cook (1954), Mühlmann and Tietz (1956), and more recently Laws and Webley (1959), but as it was not found possible to obtain adequate "clean-up" of plant extractives with these methods, an alternative one measuring the *in vivo* cholinesterase inhibition resulting from oral administration of demeton-methyl to rabbits was investigated. The results of this are reported here, and comparisons have been made with *in vitro* determinations using both rabbit and human blood.

METHODS AND REAGENTS

The method of Metcalf (1951) was found very satisfactory for human plasma cholinesterase, but unsuitable for rabbit serum. Levine *et al.* (1950) state that rabbit serum contains only one enzyme, a true cholinesterase, which hydrolyses acetylcholine. Normal rabbit serum cholinesterase levels are of the order of 10% of the levels in human plasma, consequently it was necessary to employ larger samples of rabbit serum. This resulted in un-avoidable protein precipitation during development of the ferric hydroxa-mate colour complex, and high background optical density measurements. The pH change method of Michel (1949) was accordingly tested and, with modifications, found suitable for determining cholinesterase levels. This method consists of hydrolysis of acetylcholine by the cholinesterase of the blood serum to acetic acid and choline, and the measurement of the consequent change in pH. The amount of inhibition is measured by com-paring the rate of change in pH of an inhibited blood with that of normal blood.

The method of Giang and Hall (1951), commonly used for *in vitro* determinations of anticholinesterase activity, is fundamentally the same as Michel's method. However, it was found in this study, to be only one eighth as sensitive as Michel's method and suitable only for strongly inhibiting substances.

The feeding and testing of white rabbits was carried out at the Animal Ecology Section of D.S.I.R., at Taita, and all *in vivo* testings were made with these rabbits.

The following solutions were made up as recommended by Michel (1949) for plasma testing: barbital buffer, kept in a brown bottle to prevent deterioration; acetylcholine in a brown, stoppered flask and prepared daily; 0·9% NaCl for serum dilution.

Human plasma solutions were made up from freeze dried human plasma supplied by the Wellington Public Hospital. Full strength plasma solu-tions were made up aseptically with saline, and diluted 10 times with

saline for use. Under aseptic conditions, and refrigeration, the strong solution was usable up to a fortnight during which time its cholinesterase level did not change significantly. Dilute plasma solutions were prepared daily.

Samples of demeton-S-methyl of 95% purity, demeton-O-methyl 87% purity, sulphoxide 95% purity, and sulphone 100% purity, were supplied by Farbenfabriken Bayer AG. These samples were kept in a refrigerator to minimise deterioration.

Strong solutions were made up in Analar grade ethanol, kept in a refrigerator no longer than a week and dilutions of the strong solutions in water, made up as required. Pure Rogor was supplied by Fisons Pest' Control Ltd.; pure malathion (99·6% purity) supplied by the American Cyanamid Co.; commercial malathion (40%), Fruitgrowers Chemical Co., bought locally; and demeton (70 : 30) of 96% purity supplied by Farbenfabriken Bayer AG. Solutions of these were made up similarly.

Solutions of commercial demeton-methyl, of 50% active ingredient, were supplied by H. H. York and Co. Ltd., Wellington. Strong aqueous solutions were made up weekly and dilutions made daily.

Blood samples were taken from the marginal ear vein of the rabbit. An area around the vein was shaved, the ear massaged a little with petrolatum, a drop of xylene added to the tip of the ear to dilate the vein, and a small incision in the vein made with a scalpel blade. 2–3 ml of blood was collected in a stoppered centrifuge tube, with no anticoagulant added. Excess xylene was removed from the ear with alcohol. After centrifuging the tubes of coagulated blood, the serum was poured off, weighed and diluted five times with 0·9% saline. 1 ml of diluted serum is equivalent to 0·2 g serum. These dilutions were stable for the day of preparation, but lower values were sometimes obtained next day.

Determinations of serum cholinesterase activity were made by the method of Michel (1949) at 25°C, on 1 ml of diluted serum, with 1 ml buffer pH 8·00, and 0·2 ml of 3% acetylcholine. pH measurements were made on a Cambridge bench model pH meter with a Beckman combination glass-calomel electrode. Solutions were placed in round bottom beakers of 5 ml capacity, 15 mm inside diameter. The volume of 2·2 ml adequately covered the bulb of the electrode. The beakers were held on the surface of a thermostatically controlled water bath at 25°C $\pm$ 0·1°C.

Although electrode drift was negligible, occasional spurious readings were obtained. Such errors were avoided by making pH readings approximately $\frac{1}{4}$ hourly. Plotting pH against time gave a straight line the gradient of which, expressed as $\triangle$ pH/hr, is proportional to the cholinesterase level. In Fig. 1 are shown 5 typical plots for rabbit serum. The serum with the highest gradient (0·49) is a normal serum and that with the lowest gradient, a highly inhibited serum. The plots are offset along the pH axis.

All results are the means of at least duplicate determinations. Average differences between duplicates were less than 0·01 pH units/hr. On many samples, and where differences between duplicates were greater than 0·02 pH units/hr, further determinations were carried out.

No differentiation was made between male and female rabbits.
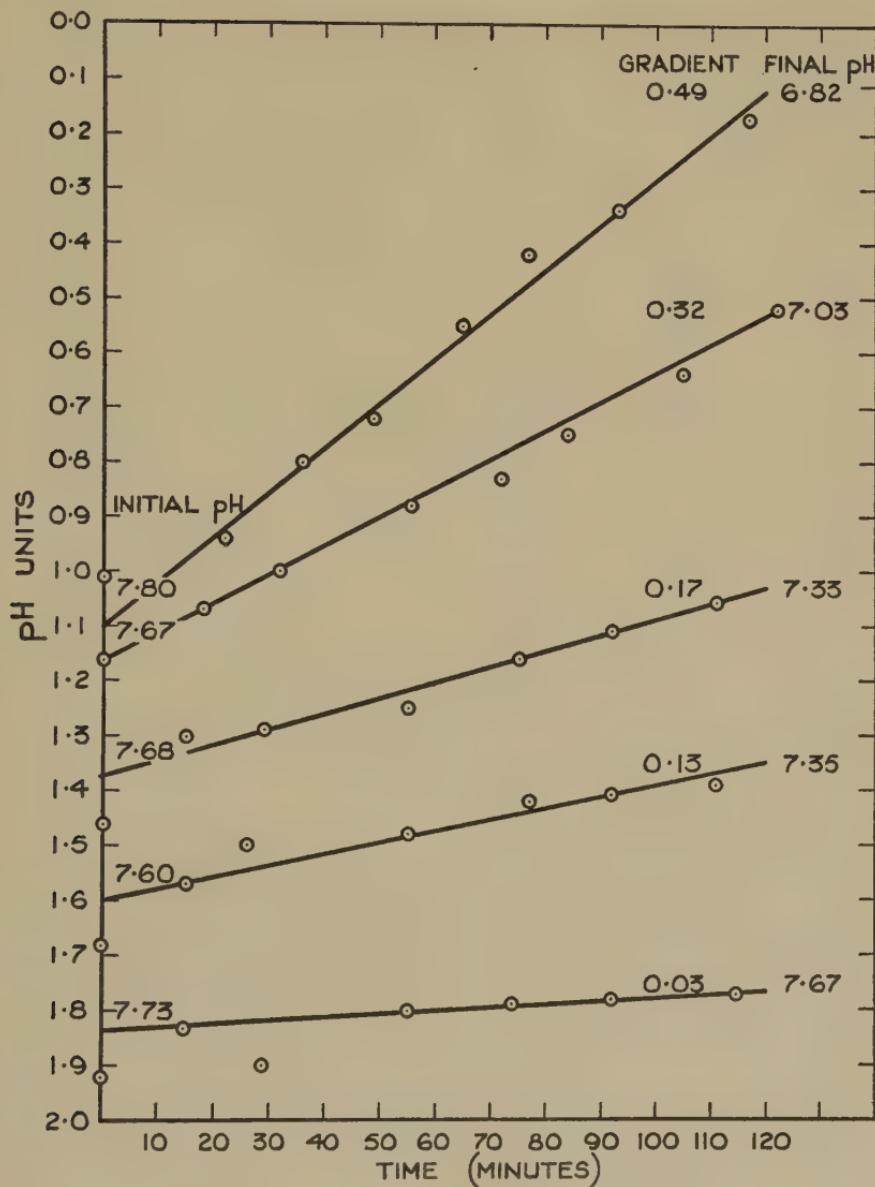
FIG. 1—pH Gradients of Cholinesterase Level Determinations on 0·2 of Rabbit Serum.

RESULTS

Normal Cholinesterase Levels

The cholinesterase levels of rabbits fed on normal diet were determined prior to all demeton-methyl dosing. The results of these determinations expressed as $\triangle$ pH/hr made over a period of several months are shown in

Sig. 6

Table 1. All figures are the average of duplicate or triplicate determinations on each sample.

Table 1—Normal Cholinesterase Values, Rabbit Blood Serum

Rabbit No.	No. of Samples Examined From Each Rabbit	Range of Cholinesterase Levels Expressed as $\triangle$ pH/hr			Standard Deviations $S = \sqrt{\dfrac{\Sigma\ d^2}{n-1}}$
		Min.	Max.	Mean	
A1♂	15	0.40	0.88	0.52	0.121
46♂	9	0.58	0.94	0.77	0.142
47♂	8	0.46	0.84	0.68	0.142
48♀	7	0.42	0.74	0.54	0.109
58♀	16	0.36	0.79	0.49	0.127
59♀	13	0.37	0.79	0.53	0.105
60♀	18	0.39	0.81	0.54	0.105
61♀	18	0.26	0.74	0.52	0.108
62♀	19	0.33	0.71	0.58	0.091
65♀	12	0.47	0.62	0.53	0.070
048♂	9	0.56	0.72	0.63	0.054

♂ = male;   ♀ = female.

The mean cholinesterase levels shown in Table 1 were taken to be the normal cholinesterase levels for each of the rabbits and were used for all calculations of *in vivo* cholinesterase level depressions.

A number of determinations of normal serum cholinesterase were made on two separate occasions on samples taken at two-hourly intervals. These results are shown in Table 2.

Table 2—Normal Cholinesterase Values, Rabbit Blood Serum, Variation During Day

Rabbit No.	Cholinesterase Value at Time		Rabbit No.	Cholinesterase Value at Time		
	0 hr $\triangle$ pH/hr	2 hr $\triangle$ pH/hr		0 hr $\triangle$ pH/hr	2 hr $\triangle$ pH/hr	4 hr $\triangle$ pH/hr
A1	0.40	0.43				
62	0.55	0.65	62	0.55	0.46	0.59
61	0.50	0.59	61	0.48	0.49	0.47
60	0.56	0.57	60	0.46	0.50	0.44
			58	0.42	0.41	0.39
			65	0.49	0.46	0.50
			59	0.54	0.45	0.37

From the figures shown in Tables 1 and 2 it is apparent that the normal cholinesterase level is randomly fluctuating rapidly from hour to hour as well as from day to day and no fixed level can be assigned to any one rabbit. The standard deviations are variable from one rabbit to another.

This fluctuation in normal cholinesterase levels made precise determination of the per cent lowering of cholinesterase levels difficult at low dosages but satisfactory at higher dosages.

Feeding Trials

Weighed amounts of demeton-methyl were diluted with water and the requisite amount added to gelatin capsules by micro pipette. Blood samples were taken immediately prior to feeding and the figures obtained for serum cholinesterase levels were used for assessing the mean level as shown in Table 1. To measure the rate of response of serum cholinesterase levels to a single oral ingestion of demeton-methyl, blood samples were taken at intervals after ingestion. The results of the trials are shown in Fig. 2. Duplicate determinations were made on the samples the same day.

Minimum cholinesterase levels occurred between 1 and 3 hours after dosing in all cases, and in later experiments the optimum time of sampling for the minimum level following a single dose was taken as 2 hours. After 48 hours the cholinesterase levels of all samples were within the standard deviation from the normal at these dosages. Between subsequent dosings, at least 3 days were allowed to elapse by which time levels were found to be within the standard deviation from the normal. Dosages are expressed as milligrams of active ingredient per kilogram of animal weight (or mg/kg).

Each of 7 rabbits was given a range of dosages of demeton-methyl and samples were then taken to determine the minimum cholinesterase levels, which were then plotted against demeton-methyl dosage, and I_{50} values (dosage required to produce 50% inhibition of cholinesterase) measured from the plots. The plots of rabbits 61 and 60, whose I_{50} values were respectively the maximum and minimum obtained, are shown in Fig. 3. Each point shown is the mean of repeated dosings, three or more being carried out where pretreatment cholinesterase values were outside the standard deviation from the normal.

The I_{50} figures obtained for all the rabbits tested are shown in Table 3.

TABLE 3—Demeton-methyl, *In Vivo* Cholinesterase I_{50} Figures for Rabbit Blood Serum

Rabbit		I_{50} (mg/kg)	Rabbit		I_{50} (mg/kg)
A1	......	7.4	58	......	7.2
59	. ....	6.6	60	......	5.7
61	......	7.4	62	......	7.0
65	......	7.4			
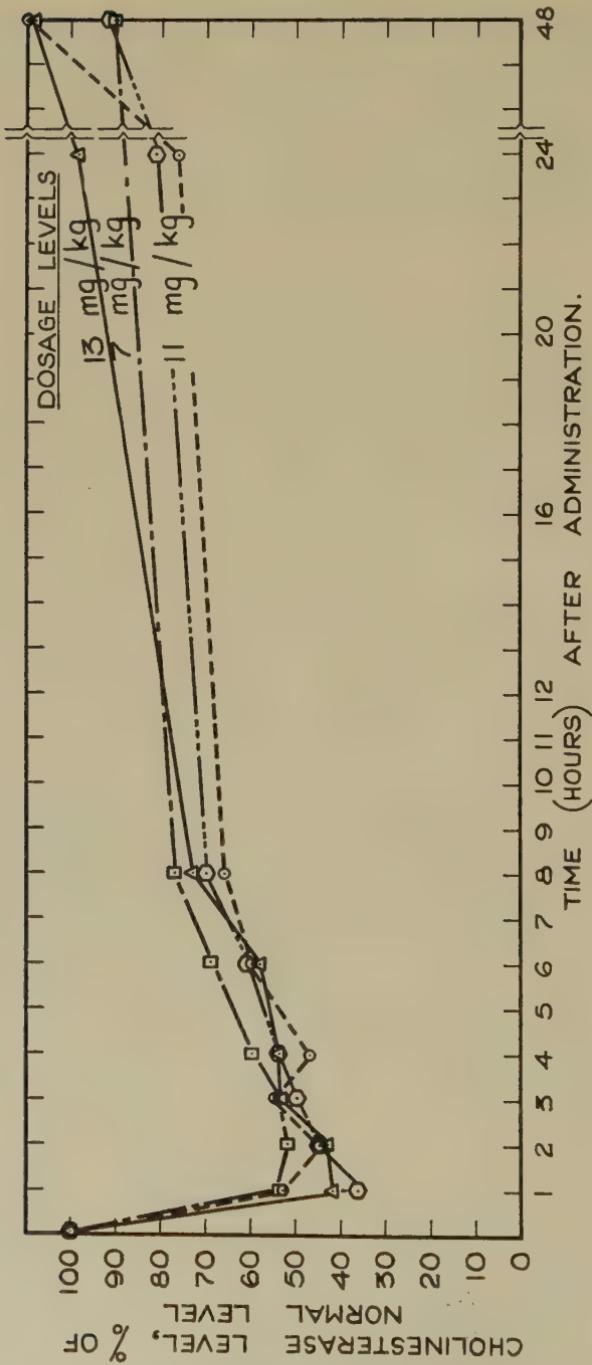
FIG. 2.—Depression and Recovery of Rabbit Serum Cholinesterase Levels after a Single Dosage of Demeton-methyl.

The average I_{50} figure was 7·0 mg/kg. At dosages above 11 mg/kg some of the rabbits developed tremors caused by acetylcholine poisoning. As the cholinesterase levels are rapidly restored, the symptoms soon disappeared. Barnes and Davies (1951) found that cholinesterase levels in animals could be lowered to 20% without symptoms of poisoning.

A comparison was made between *in vivo* and *in vitro* anticholinesterase activity in rabbit blood serum. Samples were taken for determination of normal levels, and a further 5 ml removed and placed in a 37°C water bath. Immediately, a demeton-methyl capsule was fed to the rabbit and the equivalent amount in relation to total blood volume was added to the sample in the water bath. Courtice (1943) states that the blood volume of a rabbit is 70 cc per kg. After 2 hours incubation at 37°C, the *in vitro* sample was cooled and the cholinesterase level determined. An *in vitro* level
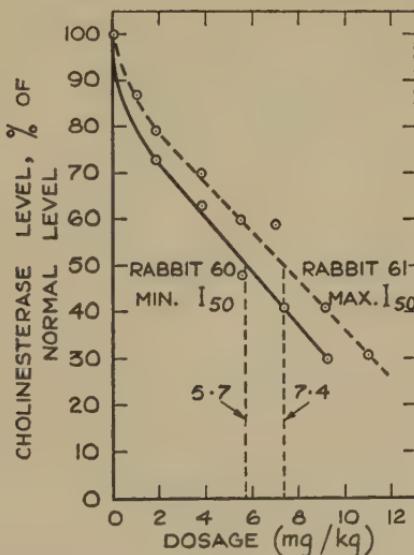
Fig. 3—Effect of Demeton-methyl Dosage Rates on the Minimum Cholinesterase Levels, Expressed as Per Cent of Normal Level.

of demeton-methyl dosage equivalent to an *in vivo* level of 7 mg/kg produced complete inhibition of the cholinesterase. Consequently, lower *in vitro* dosages were used and the conditions for the determination of I_{50} values slightly modified. 0·2 ml of pooled rabbit serum was used and the inhibition time at 37°C was reduced to 1 hr, otherwise all conditions were the same as for *in vivo* determinations. The results of the *in vitro* measurements are shown in Fig. 4.

The I_{50} value is seen to be 0·9 μg. This is equivalent to 0·32 mg/kg compared with the *in vivo* I_{50} figure of 7·0 mg/kg.

The *in vitro* inhibitory effects of demeton-methyl, demeton-S-methyl, and
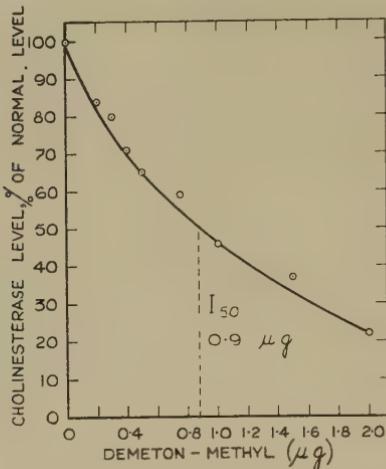
Fig. 4—The *In Vitro* Inhibition of Cholinesterase in 0·2 ml Rabbit Serum by Demeton-methyl.
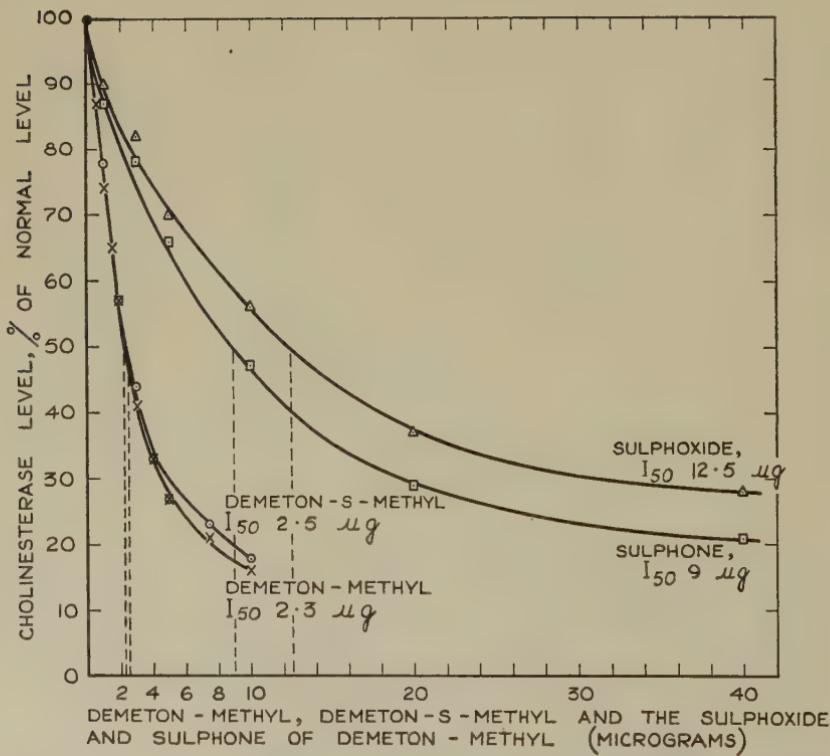
Fig. 5—*In Vitro* Cholinesterase Inhibition Produced by Demeton-methyl, Demeton-S-methyl, and the Sulphoxide and the Sulphone of Demeton-methyl in 0·02 ml Human Plasma.

the sulphoxide and the sulphone of demeton-methyl were measured on human plasma. 0·02 ml of plasma was required for analysis and the results are shown in Fig. 5.

For comparative purposes, the *in vitro* inhibitory activities of malathion (pure and commercial), Rogor (pure), demeton (70 : 30), demeton-O-methyl on human plasma, and demeton-S-methyl on human red cells were also measured. The I_{50} figures for these substances were obtained in the same way as for demeton-methyl and are shown in Table 4, together with the demeton-methyl values.

TABLE 4—Cholinesterase *In Vitro* and *In Vivo* I_{50} Figures

Substance	Source of Cholinesterase	I_{50} µg/0·2 ml Plasma, Serum, or Red Cells
Demeton-methyl	Rabbit serum, *in vivo*	20*
„ „	Rabbit serum, *in vitro*	0·9
„ „	Human plasma, *in vitro*	23
Demeton-S-methyl	Human red cells, *in vitro*	15
„ „	Human plasma, *in vitro*	25
Demeton-O-methyl	„	55
Demeton-methyl, sulphoxide meta-bolite	„	130
Demeton-methyl, sulphone meta-bolite	„	90
Demeton, 70 : 30	„	6·5
Malathion, commercial	„	500–600
Malathion, pure	„	> 5,000
Rogor, pure	„	> 10,000

*Calculated from equivalent *in vivo* value of 7·0 mg/kg.

DISCUSSION

The modification to Michel's method has made possible more accurate measurement of cholinesterase levels. If the pH gradient is derived from only the initial and final pH figures it could be considerably in error, particularly in determinations of low cholinesterase levels. Reference to Fig. 1 shows that frequently the initial pH figures are not in line with subsequent ones. The effect of spurious readings is eliminated by calculating the gradient from the plot of pH against time.

The I_{50} figure of 6·5 µg obtained in this investigation for demeton is higher than the figure of 0·8 µg (re-calculated on basis of 0·2 ml plasma) obtained by Nesheim and Cook (1959) and Yip and Cook (1959) for technical demeton. In subsequent experiments they showed that their sample had increased in toxicity to about 3 times the toxicity found when it was first tested.

Tolerances (Anon. 1960) for demeton have been based on measurement of the *in vitro* human plasma anticholinesterase activities of crop extracts using technical demeton as a standard for comparison.

In view of the change in activity of technical demeton found by Yip and Cook, and the difference between their values and that of the present investigation, it appears that technical demeton is not sufficiently uniform for use as a standard.

Due to the variation in normal cholinesterase levels of the blood of rabbits it was not possible to detect a single dose of demeton-methyl of less than 2 mg/kg. At this level, a 2·5 kg rabbit would need to be fed 1 kg of demeton-methyl treated crop material to produce a detectable cholinesterase lowering with a crop residual content of 5 p.p.m. This is quite impracticable, and it appears that residual contents of less than 50 p.p.m. could not be accurately determined by this method. *In vitro* inhibition of rabbit serum is more sensitive as a method, but suffers from the difficulties of quantitative active ingredient recovery and adequate clean-up. Work on this aspect is proceeding.

Vandekar (1958), and Heath and Vandekar (1957) have shown on the basis of increased LD_{50} figures on rats and I_{50} *in vitro* figures on sheep erythrocytes that the intravenous toxicities of highly purified isomers and metabolites of demeton-methyl are considerably increased on storage and on dilution with water due to partial self alkylation to a methyl sulphonium derivative. In this investigation, no intravenous toxicity measurements were made, but the *in vitro* I_{50} figures on rabbit serum and human plasma showed no evidence of any increased anticholinesterase activity over the period of several months during which the isomers and metabolites were examined, nor was there any significant difference between the toxicities of demeton-S-methyl and demeton-methyl. Differences in anticholinesterase activities due to impurities have been noted by Vandekar (1958) for demeton-methyl, Diggle and Gage (1951) for parathion, Cook (1955) for demeton, and is evident in the present work in the case of malathion (see Table 4).

It has been noted that a number of organo phosphorus compounds, schradan, parathion (Kilby 1954), EPN, guthion (Murphy *et al.* 1958) are weak *in vitro* inhibitors but high *in vivo* inhibitors due to metabolic conversion to active cholinesterase inhibitors. No evidence of this has been found in the present investigation on demeton-methyl, the reverse being the case. Considerable inactivation in the digestive system apparently takes place as shown by the *in vivo* I_{50} figure being 22 times the *in vitro* figure for demeton-S-methyl. This is consistent with the evidence (Heath and Vandekar 1957) that the methyl sulphonium derivative is of high intravenous toxicity and low oral toxicity. The inactivation of demeton-methyl in the digestive system of an animal may thus be an important factor in assessment of the oral toxicity of demeton-methyl.

Conclusions

1. There is a considerable degree of inactivation of demeton-methyl presumably in the digestive tract of the rabbit, which results in the *in vivo* oral toxicity of 7 mg/kg being only $1/22$ of the *in vitro* toxicity, as measured by cholinesterase inhibition. ··

2. Demeton-S-methyl and the commercial mixture demeton-methyl are of comparable anticholinesterase activity, but the sulphoxide and sulphone are of lower activities.

3. *In vivo* cholinesterase inhibition is found to be unsuitable as a method for determination of residual demeton-methyl due to the low *in vivo* toxicity of demeton-methyl.

ACKNOWLEDGMENTS

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CORE-SAMPLING BALES OF SCOURED WOOL FOR MOISTURE TESTING

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Summary

Investigations have been carried out to determine the most efficient system of core-sampling bales of scoured wool for moisture testing under N.Z. conditions. For the system developed the maximum value of the precision has been found to be ± 0·2% regain.

1. Introduction

In 1951 the N.Z. Department of Agriculture instituted a moisture testing service, whereby bales of scoured wool for export were sampled and tested for mean regain (i.e., moisture content expressed as a percentage of the dry weight) and certificates issued to enable the invoice weights to be corrected to standard regain. The geographical situation in N.Z. was a major problem as eight shipping ports and approximately twenty scouring works were scattered throughout both islands. Thus it was impossible for the complete line of wool to be tested to be taken into any one Conditioning House (as is done at the Bradford Conditioning House, for example). Nor was it practicable to have testing laboratories in every centre from which wool was shipped, because in most of them the volume of work would have been insufficient. Another major difficulty was the relatively short time between scouring and shipping during which the wool was available for testing.

These practicable considerations, which were entirely outside the control of the Department of Agriculture, had a great influence on the system which was finally put into operation (and which is still functioning satisfactorily). There are two Wool Testing Laboratories, one in Auckland and one in Wellington, in both of which centres there is a large volume of wool to be sampled and tested. In the other six ports, the Department's Sheep and Wool Instructors are available at short notice to draw samples from any lines which are required to be tested. Samples drawn for testing are immediately sent airfreight, in sealed double plastic bags, to one or other of the Testing Laboratories, where they are oven-tested in the usual way, regains calculated and certificates issued.

The only period when bales of wool are available for sampling is sometime between being pressed at the scouring works and dumped at the dumping store, immediately prior to shipment. This is narrowed down still further in practice, by the fact that the bales must be weighed at the same time as they are sampled. There are thus only two occasions on which they are available for convenient and economical sampling; at the scouring works

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when they were being weighed as they leave for the wool store, and at the dumping store when they are being weighed immediately before dumping and shipping. In practice, most sampling is carried out at the dumping stores and this is the preferred arrangement. However, in some cases this is not possible and sampling must be done at the scouring works.

Thus the sampling method used must be rapid in order that the work of the dumping store, or scouring works, is held up for the minimum possible time. It must also require as few operators as possible so that sampling is not too costly, and it must be capable of drawing a sample with a regain representative of the mean value for the line being tested. The well known methods of hand sampling were considered, but proved to be completely impracticable on press-packed bales and were also far too slow. The only known method which could fulfill the requirements was the core-boring method developed in the U.S.A. for rapid sampling of bales of greasy wool for yield determinations. Although a considerable amount of work had been done in the U.S.A. on core-boring (A.S.T.M., 1954; Cameron, 1951; Cronin, 1947; Johnston, 1949; Michelson, Tanner, and Wollner, 1942; Tanner and Deming, 1949; U.S. Dep. Agric., 1949; U.S. Dep. Agric., 1956; Wollner and Tanner, 1941), it was considered necessary to carry out investigations on the method as applied to moisture testing under N.Z. conditions of scoured wool.

2. CORE-SAMPLING EQUIPMENT

The main requirements for the equipment of an efficient sampling system are that—

(i) The sample be of adequate size for accurate oven-testing.

(ii) Minimum time and effort be required to draw core-samples by the minimum number of operators.

(iii) Cutters be easily sharpenable without special equipment.

(iv) All the equipment be robust and trouble-free.

(v) There be minimum damage to wool and packs.

Initial work on the design of equipment for N.Z. conditions was carried out by J. E. Duncan, Wool Supervisor of the N.Z. Department of Agriculture, and the equipment finally decided upon consists of a $\frac{5}{8}$ in. portable electric drill driving (at aproximately 500 r.p.m.) a removable 18 in. long steel tube of 1 in. internal diameter, having a plain (i.e. non-serrated) hardened-steel cutter screwed on to the end. A length of $\frac{3}{4}$ in. wooden dowel is used to extrude the core of wool from the tube into a double plastic bag which remains covered until sampling is completed and is then sealed.

With this equipment, cores of about 25 g each can be drawn from bales weighing 250 to 300 lb. One operator can draw at least 100 such cores per hour, without any undue physical effort. The cutters can easily be kept sharp by using a carborundum stone after every 20 to 50 cores. Two or three times a year the cutters are returned to the makers for regrinding to remove the rounded shoulder which inevitably develops from hand sharpening. The whole of the equipment has proved to be adequately robust and trouble-

free under the conditions of use, although some care is needed to prevent chipping of the cutting edge. The damage done per core to the packs is negligible, and is confined to cap and base only, as coring must be through compression surfaces in press-packed bales in order to obtain adequate weight of sample. The damage done to the wool remaining in the bale can be calculated by means of a formula derived by Michelson, Tanner and Wollner (1942). It is found that if a 300 lb bale of wool whose average staple length is 4 in. is sampled with this equipment, the amount of cut fibres of length 1 in. and less will not exceed half an ounce for each core drawn.

3. USE OF COMPOSITE SAMPLES

Very little, if any, advantage can be gained by determining the mean regain of each individual bale of a line and such a procedure is generally impracticable. Testing must therefore take place on composite samples. An important factor when composite samples are used, is the relation between core weight and total bale weight. The weights of the bales in a line of scoured wool can vary quite markedly (e.g., a range of 100 lb is not uncommon), so that for a composite sample to be representative of a line, the weights of the samples from the individual bales should be proportional to the weights of the bales. In general, core-sampling N.Z. bales of scoured wool with the equipment described in the previous section, has been found to be completely satisfactory in this regard.

The main object of these investigations was to design a system of core-sampling (i.e., determine the way in which the cores should be drawn from a bale, the number of cores to be taken from each bale and the number of bales to be sampled for various sizes of line), which would give a composite sample with a regain as near as possible to the true mean regain of the whole line. The system also had to fulfill the practical requirements already enumerated.

4. DISTRIBUTION OF MOISTURE IN A BALE

The ways in which moisture can be distributed throughout a bale of wool is of obvious importance to any study of methods of obtaining samples with a mean regain representative of the whole bale. Accordingly tests were made by core-sampling from various positions in bales, and measuring regains of the individual cores. It was necessary to have some means of testing samples of about 25 g and to do this a small four-unit drier was built in which four separate core-samples could be dried at the same time under controlled conditions. This drier was of the forced-draught type, the samples being placed in tared metal cans which could be inserted into the stream of hot air for 30 min. and sealed off, removed from the drier, allowed to cool and weighed on an analytical balance. The temperature of the air being forced through the sample was electronically controlled at 105°C $\pm$ $\frac{1}{2}$°C and the flow of hot air through each of the four samples was controlled at a standard value by means of manually operated valves. Tests

on this drier showed that it gave repeatable results, although regain values determined with it were 0·6% regain lower than those obtained on identical samples in the conditioning oven used for routine moisture tests and all regains quoted have been corrected to allow for this difference.

Using this apparatus, several bales were tested by taking a number of equally spaced, parallel cores from one end of the bale, as shown in Fig. 1.
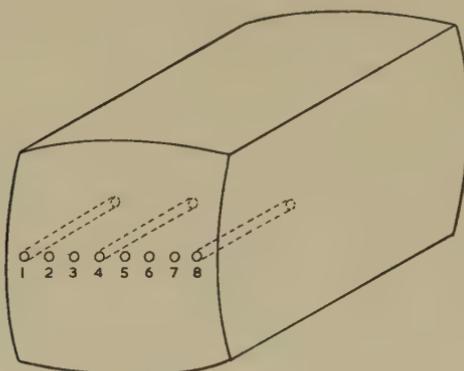

FIG. 1—Experimental core positions.

Each core was tested for mean regain and the results so obtained were all of the same general form as that shown in Fig. 2, where regain has been plotted against core position. Thus the cores taken from nearest the edges had higher regains than those taken from more central positions.

If the bale is considered as being made up of concentric layers of wool, it will be seen that the cores from the central positions take a much greater
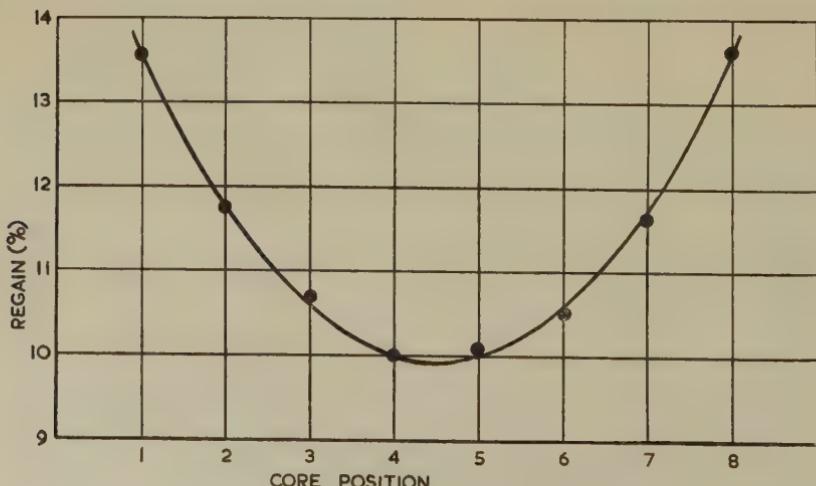
FIG. 2—A typical result of an experimental sampling as in Fig. 1.

proportion of wool from the deeper layers than do the cores taken from the outer positions. Thus the most likely explanation for the results obtained, is that the inside layers had lower regains than the outer ones, i.e., the bales had moisture gradients from the outside in to the centre. This is consistent with the known hygroscopicity of wool and the fact that at this time (1951 to 1953) scourers in N.Z. generally overdried their wool. Thus as soon as the wool was baled, the outer layers would begin to take up water vapour from the atmosphere and a moisture gradient would develop.

To investigate the form of these gradients, the procedure was adopted of dividing core-samples into several small sections and measuring the regains of each sub-core. With the drier used the gradients could only be checked by taking four cores as close together as possible, dividing each into four sub-cores and testing sub-cores corresponding to same depth in the bale as one sample representing that depth. However, it was soon felt desirable to be able to determine the regain values at more than four depths in the bale and apparatus was designed in which 2·5 g sub-samples could be tested quickly. This consisted, essentially, of an infra-red lamp with beam directed on an auxiliary pan of an analytical balance. With this equipment it was possible to obtain regains at approximately ten points along a core, thus giving a better indication of the form of the moisture gradient in the bale.

A large number of bales were tested in this way and it was found that in most cases the gradients were of the general form shown in Fig 3. This again corresponds to the case of a bale which has been somewhat overdried and then stored in a moist atmosphere. Some bales were found to have gradients the inverse of this form, these corresponding to the case of under-dried wool losing moisture to the atmosphere. Several bales were tested almost immediately after being pressed, and in these there was no appreciable gradient, as would be expected. In a very few instances bales were found in which there were rather more complex moisture distributions. These bales were mostly of high mean regain (e.g., 19%) and had been in the
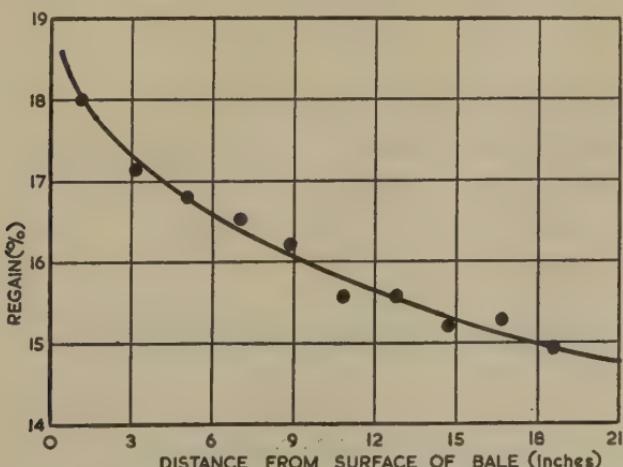
FIG. 3—A typical moisture gradient in a core from the centre of the cap of a bale.

store for several months, which is much longer than the usual storage period. During this time the relative humidity and temperature in the store would probably have gone through several complex cycles. Slight irregularities were often found in the gradients, almost certainly due to uneven initial drying at the scouring works.

Although the general form of the moisture gradients was always found the same in cap and base of the same bale, it was common to find that these two halves had slightly different mean regains, the difference being, of the order of 0·5% regain. For overdried wool, the cap usually had the higher value, and conversely for underdried wool. The most likely explanation of this lies in the method of storage. Bales are usually stood on their base ends (in order that the brand on the cap may be easily seen) and are packed as closely together as possible. Thus the cap is the part of the bale which is most readily accessible to the atmosphere and a greater gain or loss of water vapour should occur at the cap.

5. Calculations on Coring Positions

Following the establishment of the general form of moisture gradients occurring in bales of scoured wool, it became possible to calculate the positions from which cores should be taken to obtain samples which theoretically have mean regains equal to the mean value of the whole bale.

A bale of scoured wool which has been well pressed is of a shape difficult to define mathematically. The nearest geometrical solid would be a prolate spheroid, but for simplicity the bulges can be ignored and a bale considered as being a rectangular solid with centre at the origin and sides of lengths $2a$, $2b$, $2c$.

The general form of the experimentally found gradients, both for overdried and underdried wool, is then given, to a fair approximation, by the equation

$$\frac{M - M_s}{M_0 - M_s} = C \cos \frac{\pi x}{2a} \cos \frac{\pi y}{2b} \cos \frac{\pi z}{2c} \qquad \text{.....................(1)}$$

where M = regain at any point (x, y, z) of the bale.

M_s = regain at all points on the surface of the bale, i.e., at $x = \pm a$, $y = \pm b$, $z = \pm c$.

M_0 = regain at the centre of the bale, i.e., at $x = y = z = 0$.

C = a constant.

Now, the mean regain of the whole bale, $\bar{M}$, is

$$\bar{M} = \frac{1}{8abc} \int_{-a}^{a} \int_{-b}^{b} \int_{-c}^{c} M \, dx \, dy \, dz \qquad \text{.....................(2)}$$

Thus we have

$$\frac{\overline{M} - M_s}{M_0 - M_s} = \frac{1}{8abc} \int_{-a}^{a} \int_{-b}^{b} \int_{-c}^{c} C \cos\frac{\pi x}{2a} \cos\frac{\pi y}{2b} \cos\frac{\pi z}{2c} \, dx \, dy \, dz \quad \text{...............(3)}$$

$$= \frac{8C}{\pi^3} \quad \text{....................(4)}$$

We can now consider cylindrical core-samples taken from various positions in this bale and calculate their mean regains. These values can then be compared with the value of $\overline{M}$ given in equation (4). In this way we can determine from which positions in the bale core-samples should be taken to obtain the most accurate estimate of $\overline{M}$.

The position which obviously would be the one most easily and accurately located in practice, would be from the centre of either cap or base of the bale, taking the core parallel to the axis of the bale. For this case we can calculate to what depth the coring tube must penetrate for the mean regain of the sample, $\overline{m}_1$, to be equal to the whole-bale mean, $\overline{M}$.

Let the tube penetrate to a depth v, then

$$\frac{\overline{m}_1 - M_s}{M_0 - M_s} = \frac{1}{v} \int_{a-v}^{a} C \cos\frac{\pi x}{2a} \, dx$$

$$= C\frac{2a}{v\pi} \left(1 - \cos\frac{\pi v}{2a} \right) \quad \text{..............(5)}$$

Now for $\overline{m}_1 = \overline{M}$, we must have

$$\frac{\overline{m}_1 - M_s}{M_0 - M_s} = \frac{\overline{M} - M_s}{M_o - M_s}$$

so that

$$\frac{2a}{v} \left(1 - \cos\frac{\pi v}{2a} \right) = \frac{8}{\pi^2} \quad \text{....................(6)}$$

Solving this numerically for v/a we find

$$\frac{v}{a} = 0{\cdot}34 \quad \text{................(7)}$$

Sig. 7

Thus for such a centre-core to give an accurate sample, it must only penetrate approximately one-third the distance in to the middle of the bale. Taking the average dimensions of a bale as 45 in. $\times$ 28 in. $\times$ 28 in., this means that the coring-tube should be only $7\frac{1}{2}$ in. long for centre-coring.

It is rather unfortunate that this investigation was not carried out before the design of the sampling tubes was decided upon and a considerable number of the 18 in. long $\times$ 1 in. diameter tubes manufactured. However, such was the case and for reasons of cost it was necessary to continue using these tubes if at all possible. Thus centre-coring was not possible in practice.

Consider now a core of length $4a/5$ (i.e. 18 in. for $2a = 45$ in.), taking parallel to the X-axis but displaced a distance d from it along either the Y or Z axis. We then obtain a core with a mean regain, $\bar{m}_2$, given by

$$\frac{\bar{m}_2 - M_s}{M_0 - M_s} = \frac{5}{4a} \int_{a/5}^{a} C \cos \frac{\pi x}{2a} \cos \frac{\pi d}{2b} \, dx$$

$$= C \frac{5}{2\pi} \cos \frac{\pi d}{2b} \left(1 - \sin \frac{\pi}{10} \right) \quad \dots\dots\dots(8)$$

For $\bar{m}_2 = \bar{M}$ we have

$$\frac{5}{2} \cos \frac{\pi d}{2b} \left(1 - \sin \frac{\pi}{10} \right) = \frac{8}{\pi^2} \quad \dots\dots\dots(9)$$

Solving this numerically for d/b we find

$$\frac{d}{b} = 0.69 \quad \dots\dots\dots(10)$$

This result will hold for both $M_0 < M_s$ (i.e. a bale taking up moisture from the atmosphere), and $M_0 > M_s$ (i.e., a bale losing moisture to the atmosphere).

In practice, of course, the assumptions made above do not strictly apply. Bales of wool are not simple rectangular solids and do not have moisture distributions of the exact mathematical form considered above (due to uneven drying of the wool, methods of storage before and after packing, etc.). However, the gradients found experimentally in bales of scoured wool do approximate the form of expression (1), and there is also the geometrical fact that approximately 75% of the volume of a bale of wool is contained in the outer 6 in. layer. It would, therefore, seem that to obtain a representative sample using an 18 in. long coring tube, the core should be taken from either end of the bale, parallel to the sides, 4 in. in from the seam, and equidistant from two opposite sides.

6. POSITIONING ERRORS

If this method were used in practice for routine sampling, it would be impossible to ensure that each core was drawn in exactly the way described above. Thus an important consideration is the effect of positioning errors, i.e., the differences between mean-bale-regain and mean-core-regain arising from the cores being taken from positions differing from the optimum one. For example, the core may be taken parallel to the sides and midway between two opposite sides, but either too near the seam or too far from it. Calculations have been made on these effects for a hypothetical case of a rectangular bale with a moisture gradient similar to that shown in Fig. 3, and with a mean regain ($\bar{M}$) of 18%. (This is somewhat higher than the usual value of M, so that the following values of positioning error will be, if anything, greater than those which normally occur.) For this case, the errors, in per cent regain, resulting from typical incorrect positionings of the core are as follows:

Core parallel to bale axis, in XY plane, 2 in. from seam;   error $= + 0.6\%$
Core parallel to bale axis, in XY plane, 3 in. from seam;   error $= + 0.4\%$
Core parallel to bale axis, in XY plane, 5 in. from seam;   error $= - 0.2\%$
Core parallel to bale axis, in XY plane, 6 in. from seam;   error $= - 0.4\%$
Core parallel to bale axis, $\pm$ 1 in. from XY plane, 4 in. from seam; error $= + 0.01\%$
Core parallel to bale axis, $\pm$ 2 in. from XY plane, 4 in. from seam; error $= + 0.03\%$
Core 10° to bale axis, in XY plane, 4 in. from seam;   error $= - 0.3\%$
Core 20° to bale axis, in XY plane, 4 in. from seam;   error $= - 0.6\%$

Any combination of the above three types of positioning errors could occur. It will be seen from the above data that in many cases such combinations could lead to a reduction in the regain error. For example, if the core were taken 2 in. from the seam, 2 in. from the XY plane, and at 20° to the bale axis, the resulting error would be $+ 0.03\%$ regain.

Two conclusions can be drawn from this information. Firstly, less care is needed in ensuring that the core is equidistant from two opposite sides of the bale than in ensuring that it is the correct distance from the seam and parallel to the bale axis. Secondly, it is better to take the core slightly more than 4 in. from the seam than slightly less than 4 in. from the seam. These points were consequently emphasised in the instructions issued to all sampling-officers, and it has been found that after a little practice they can position their cores quite satisfactorily.

7. ACCURACY OF THE THEORETICAL SAMPLING METHOD

To determine the accuracy of the sampling procedure described above, it was first necessary to be able to obtain an accurate estimate of the mean regain of a whole bale, so that a test could be made for bias in the samples obtained. The method used was to stratify each end of the bale into 16 equally sized sections and draw one core from a random position in each

section with a 24 in. long, 1 in. diameter tube, which could penetrate right to the centre of the bale. In this way 16 cores were obtained from each of cap and base, to form a 32 core composite sample having a mean regain which was taken as an estimate of that of the whole bale. To check on this, a bale which was so sampled was accurately weighed and then the total dry weight of the wool was obtained by drying it in a conditioning oven, approximately 3 lb at a time. It was found that the composite sample had a mean regain within $\pm$ 0·1% regain of the whole bale mean value.

Tests were made on a large number of bales from various scouring works throughout N.Z. From each bale a composite stratified random sample of 32 24 in. long cores was drawn as described above, to obtain an accurate estimate of the whole-bale mean regain. In addition, 50 cores were drawn from each of cap and base using the usual 18 in. long tubes. Four of these cores were drawn from the theoretical positions and the remainder from positions selected at random. Each individual 18 in. long core was tested for mean regain (and one each from cap and base for moisture gradient). By plotting the core-regains on probability paper (Brooks and Carruthers, 1953), it was found that, for both sets of 18 in. long cores, the regains were normally distributed about their mean values. Further in no case did the mean values differ significantly from the estimates of the corresponding whole-bale means.

Using the differences between the whole-bale means and the core regains in an analysis of variance, showed that the regains of the cores taken from positions selected at random in cap and base of a bale, had a within-bale standard deviation of approximately 1% regain. For the cores taken approximately parallel to the sides, about 4 in. in from the seam and roughly equidistant from two opposite sides, the within-bale standard deviation was 0·5% regain. The value of the between-bale standard deviation was approximately 1·7% regain. A few earlier tests had indicated that this quantity was of the order of 1% regain, its value, is, however, of no great importance for the particular sampling system developed here, as will be shown in the next section.

8. Sampling of Multiple Bale Lines

Consider a line of N bales, having an overall mean regain $\bar{\bar{M}}$. If this line is sampled by taking k cores from each of n bales, then the composite sample of nk cores will have a mean regain $\bar{\bar{m}}$, which will differ from $\bar{\bar{M}}$ by an amount determined by the precision for the particular sampling system (Cameron, 1951). At a given probability p, the 100 $(1-2p)$ confidence limits for $\bar{\bar{m}}$ about $\bar{\bar{M}}$ will be $\bar{\bar{m}} \pm E$, where E is the precision for probability p. If the cores from a bale have regains which are normally distributed about the mean value $\bar{m}_1$ for that bale, and if the mean values for all the bales are normally distributed about the line mean $\bar{\bar{M}}$, then

$$E = t_\sigma \atop \overline{m} \qquad\qquad ....\,.......\,\,..(11)$$

where t is the normal deviate exceeded with probability p, and $\sigma_{\overline{m}}^2$ is the variance of $\overline{m}$.

Now
$$\sigma_{\overline{m}}^2 = \frac{N - n}{N} \cdot \frac{\sigma_b{}^2}{n} + \frac{\sigma_w{}^2}{nk} \qquad\qquad ....(12)$$

where $\sigma_b{}^2 = $ the variance of the mean regains of the bales in the line

$\sigma_w{}^2 = $ the variance of the mean regains of cores from the same bale.

Therefore
$$E = t \sqrt{\frac{N - n}{N} \cdot \frac{\sigma_b{}^2}{n} + \frac{\sigma_w{}^2}{nk}} \qquad\qquad ....(13)$$

From this we can determine the value of E at any given probability for any sampling system (i.e., for any values of N, n, and k), provided we know the appropriate values of σ_w and σ_b. We may take σ_b as 1% regain, and for σ_w we can use the value of 0·5% regain quoted in the previous section.

Table 1 gives the values of E in per cent regain, at a statistical probability of 0·95 (for which $t = 1·960$), for various sampling systems, taking $\sigma_w = 0·5\%$ regain and $\sigma_b = 1·0\%$ regain. It is assumed that:

(i) The n bales are selected at random from the entire N bales comprising the line.

(ii) The cores are drawn from caps and bases at random.

(iii) The positions from which the cores are drawn are selected at random, within the provisions of the method described in section 5.

8. 1 Number of Bales to be Sampled (n)

These figures show that when every bale of a line is not sampled (i.e., $n < N$), a greater total number of cores must be taken to obtain a given sampling precision than when every bale is sampled (i.e., $n = N$). For example, if a 15 bale line is sampled by taking two cores from every bale, giving a total of 30 cores, Table 1 shows that $E = \pm 0·18\%$ regain. To obtain the same value of E when only 14 of the 15 bales are sampled, 5 cores must be taken from each of these 14 bales, giving a total of 70 cores, so that over twice as much work is involved in carrying out the sampling. Also, although the value of σ_w is not likely to exceed 0·5% regain, the value of σ_b could quite possibly be greater than 1% regain. In this case, the values of E would be greater than those shown in all but the first section of Table 1, so that it would be quite possible that even more than 70 cores would be required in the above example.

Table 1—Precision E, for Different Sampling Systems

N		5	10	15	20	25	30	40	50	100	200
	n	5	10	15	20	25	30	40	50	100	200
k											
1		0.45	0.32	0.26	0.22	0.20	0.18	0.16	0.14	0.10	0.07
2		0.32	0.22	0.18	0.16	0.14	0.13	0.11	0.10	0.07	0.05
3		0.26	0.18	0.15	0.13	0.12	0.11	0.09	0.08	0.06	0.04
4		0.22	0.16	0.13	0.11	0.10	0.09	0.08	0.07	0.05	0.04
5		0.20	0.14	0.12	0.10	0.09	0.08	0.07	0.06	0.04	0.03
	n	—	9	14	18	23	27	36	45	90	180
k											
1		—	0.39	0.30	0.28	0.23	0.22	0.20	0.18	0.12	0.09
2		—	0.32	0.23	0.22	0.19	0.18	0.16	0.14	0.10	0.07
3		—	0.29	0.21	0.20	0.17	0.16	0.14	0.13	0.09	0.06
4		—	0.27	0.19	0.18	0.16	0.16	0.13	0.12	0.08	0.06
5		—	0.26	0.18	0.18	0.15	0.15	0.13	0.12	0.08	0.06
	n	4	8	12	16	20	24	32	40	80	160
k											
1		0.67	0.47	0.39	0.34	0.30	0.27	0.24	0.21	0.15	0.11
2		0.57	0.40	0.33	0.29	0.25	0.23	0.20	0.18	0.13	0.09
3		0.53	0.38	0.31	0.27	0.24	0.22	0.19	0.17	0.12	0.08
4		0.51	0.36	0.30	0.26	0.23	0.21	0.18	0.16	0.11	0.08
5		0.50	0.35	0.29	0.25	0.22	0.20	0.18	0.16	0.11	0.08
	n	—	7	11	14	18	21	28	35	70	140
k											
1		—	0.56	0.43	0.40	0.34	0.32	0.28	0.25	0.17	0.13
2		—	0.49	0.38	0.35	0.30	0.28	0.25	0.22	0.16	0.11
3		—	0.47	0.36	0.33	0.28	0.27	0.23	0.21	0.15	0.10
4		—	0.46	0.35	0.32	0.28	0.26	0.23	0.20	0.14	0.10
5		—	0.45	0.34	0.32	0.27	0.26	0.22	0.20	0.14	0.10
	n	3	6	9	12	15	18	24	30	60	120
k											
1		0.93	0.66	0.54	0.47	0.42	0.38	0.33	0.29	0.21	0.15
2		0.84	0.59	0.48	0.42	0.37	0.34	0.30	0.26	0.19	0.13
3		0.80	0.57	0.46	0.40	0.36	0.33	0.28	0.25	0.18	0.13
4		0.79	0.55	0.45	0.39	0.35	0.32	0.28	0.25	0.18	0.12
5		0.77	0.55	0.45	0.39	0.35	0.32	0.27	0.24	0.17	0.12
	n	2	5	8	10	13	15	20	25	50	100
k											
1		1.30	0.77	0.60	0.55	0.47	0.39	0.35	0.34	0.24	0.17
2		1.20	0.71	0.54	0.50	0.43	0.41	0.35	0.30	0.22	0.15
3		1.17	0.68	0.52	0.48	0.42	0.38	0.34	0.29	0.21	0.15
4		1.15	0.67	0.51	0.47	0.41	0.36	0.34	0.29	0.20	0.14
5		1.14	0.66	0.51	0.47	0.40	0.36	0.33	0.29	0.20	0.14

The effects of σ_b can be eliminated entirely, if every bale is sampled for all sizes of line. In this case equation (13) reduces to

$$E = \frac{t\sigma_w}{\sqrt{Nk}} \qquad\qquad ....(14)$$

and the first section of Table 1 applies.

As pointed out earlier, conditions in N.Z. necessitate the minimum possible time being required for sampling, so that the obvious system to be used is one of core-sampling every bale of all lines. Accordingly, this was made the basis of the N.Z. Department of Agriculture's sampling system for routine moisture testing.

8. 2 Number of Cores per Bale (k)

It can be shown (Deming, 1950) that the most economical sampling is achieved when k is taken as the nearest whole number to k_e, which is given by

$$k_e = \frac{\sigma_w}{\sigma_b} \sqrt{\frac{C_1}{C_2}} \qquad\qquad ....(15)$$

where C_1 is the approximate cost of positioning a bale for sampling and C_2 is the approximate cost of drawing one core from a bale.

Under N.Z. conditions $\sigma_w \leqslant \sigma_b$ and $C_1 < C_2$, so that the most economical sampling is achieved by taking $k = 1$. This is also one of the basic points in the sampling system used. However, there are two factors which affect this result in practice, for certain values of N. Firstly, a certain minimum weight of composite sample (e.g. 500 g) is required to carry out an accurate moisture test by the oven method, so that more than one core averaging 25 g must be drawn from each bale of the smaller sized lines. Secondly, Table 1 shows that E becomes rather large for the smaller lines (e.g., $N < 25$) when only one core is drawn per bale. It is therefore necessary to take a greater number of cores per bale as the size of line decreases below 25 bales.

9. SAMPLING SYSTEM USED

We can now describe the actual sampling system which was derived from these investigations and is used for all routine sampling.

When a line of scoured wool which requires testing is ready to be weighed at the dumping store (or in certain circumstances, at the scouring works), arrangements are made for an officer of the Department of Agriculture to be on hand with his sampling equipment. Either immediately before or immediately after each bale has been weighed, at least one full-depth core is taken from it with an electrically-rotated tube, 18 in. long and 1 in. internal diameter. The cores are taken from cap or base only, approximately parallel to the sides of the bale, roughly midway between two opposite sides, and about 4 in. in from the seam on one edge, the particular edge being chosen at random. For lines having one core taken per bale, caps

and bases are sampled alternately, the bales being brought to the scales in random order, so that as near as possible equal numbers of cores are obtained from caps and bases. For lines having more than one core taken per bale, the cores are divided as equally as possible between caps and bases. For example, if 3 cores are to be taken from each bale, two will be taken from one end (say cap) and one from the other (base) of the first bale, and vice versa for the next bale, and so on alternately. As soon as the tube is withdrawn from the bale, it is removed from the drill and the core of wool is extruded with a wooden rod into a double plastic bag, the opening of which is kept covered at all times. Immediately the line is completed. or the bag is full (up to 50 cores are contained in one double bag), the two bags are sealed independently by tightly twisting the neck, doubling it over and fastening with rubber bands. This composite sample is then placed in a strong canvas bag, along with the necessary documents, and sent to the laboratory for testing.

The number of cores, k, taken from each bale for various sizes of line, N, is shown in Table 2, together with the corresponding values of the precision, E, calculated from equation (14).

TABLE 2—Sampling Schedule

N		1	2	3	4	5	6	7	8	9–12	13–24	25–1000
k		25	13	9	7	5	5	4	4	3	2	1
E		0.20	0.19	0.19	0.19	0.20	0.18	0.19	0.17	0.19–0.16	0.19–0.14	0.20–0.03

The maximum value of E is seen to be $\pm$ 0·20% regain, and occurs for $N = 1$, 5 and 25. For all other sizes of line, E is less than 0·20% regain. In fact, Fig. 4 shows that as N increases above 25 bales, E steadily decreases. This is inevitable when every bale in a line is sampled and is an advantage in that it compensates to some extent for the decrease in accuracy in terms of pounds weight of the corrected invoice weights for the larger lines. Fig. 4 shows that whereas the probable error in the corrected invoice weights changes only slightly with N for this sampling system (Curve 2), if a system was used in which $E = 0·20\%$ regain for all values of N, it would increase in direct proportion to N (Curve 3). This may not seem of much consequence, but it should be remembered that the only point of interest to the persons paying for and receiving the certificates based on these tests, is the corrected invoice weight of the line. A difference in accuracy of ten or more pounds weight is of definite importance.

10. CONCLUSIONS

Repeat tests on many lines have all indicated that this sampling system does give the order of accuracy quoted above, which is considered to be quite adequate for practical purposes. To obtain lower sampling errors, it would be necessary to take more cores from each bale. For example, to
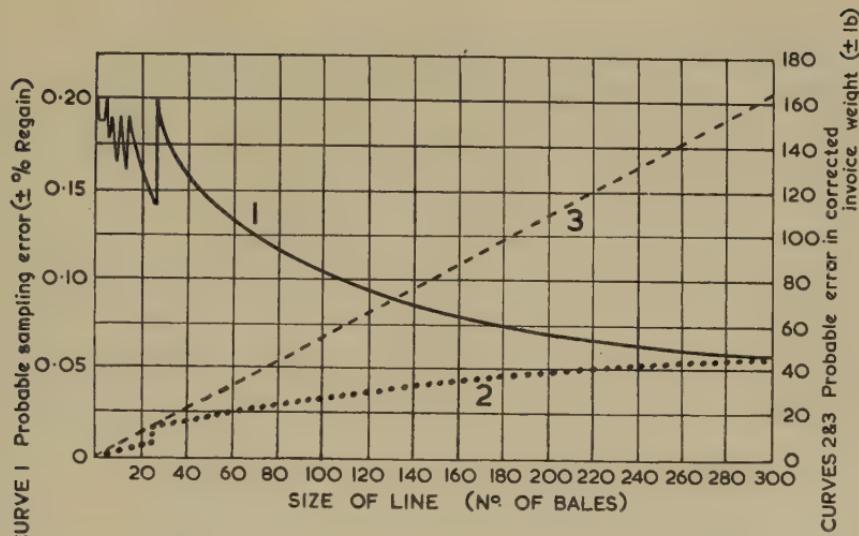
Fig. 4—Sampling errors at 0·95 probability for different sizes of line. Curve 1 shows the variations of E with N for the sampling system described. Curve 2 shows the resulting error in the corrected invoice weight for bales of 270 lb average net weight. Curve 3 shows the error in the corrected invoice weight resulting from a sampling system having $E = 0·20\%$ regain for all values of N.

reduce the value of E for $N = 25$ (the most common size of line dealt with in N.Z.) from $\pm$ 0·20% to $\pm$ 0.12%, one core would have to be taken from each of cap and base of every bale, giving a total of 50 cores. To reduce E still further to $\pm$ 0·08% regain, two cores would have to be taken from each end, making a total of 100 cores from the 25 bales. For routine sampling under N.Z. conditions, the extra time required to do this additional coring would be completely prohibitive.

Acknowledgments

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A NOTE ON FISHES FROM THE ROSS SEA, ANTARCTICA

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Summary

Identifications are made of ninety-two fishes (including one from the stomach of another fish) obtained during the N.Z. Oceanographic Institute survey of the Ross Sea in January 1959. The distribution of these according to station is given.

INTRODUCTION

From 7 January to 27 January 1959 an oceanographic cruise was conducted in the Ross Sea by the N.Z. Oceanographic Institute on HMNZS *Endeavour*. Biological work both benthic and pelagic in nature was carried out (Bullivant, 1959). The writer joined the scientific party for the period of this cruise and made field collections as part of a programme of Biological Research sponsored by the Committee on Polar Research of the National Academy of Sciences, Washington, and directed by Dr R. G. Miller of Long Beach State College. During this time twenty-one stations were occupied. Three other stations were occupied between 29 January and 6 February 1959 after the writer had left the vessel at McMurdo Sound. No additional fish were taken at these stations.* The fishes of this expedition were for the most part loaned to the Long Beach State College group for study.

The twenty-four stations were well distributed over approximately 1,000 square miles of the Ross Sea and the depths sampled ranged from 64 to 2,380 metres. The fish mentioned in this paper were secured at twelve of these stations.

EQUIPMENT AND FIELD METHODS

Bottom samples were taken with two agassiz trawls and a naturalist's dredge. The agassiz trawls, referred to as T.P. and T.A.S., measured about the mouth 18 in. by 40 in., and 10 in. by $32\frac{1}{2}$ in. respectively. The naturalist's dredge (D.N.) measured 7 in. by 20 in.

The metre plankton net used for vertical plankton hauls took a small chaenichthyid on one occasion.

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*Nine fish were collected by Dr R. K. Dell, Dominion Museum, Wellington, from *Endeavour*, off McMurdo Sound, and of six more fish taken at the ice edge, five were *Trematomus hansoni*.

Table 1—Date, Depth, and Number of Fish Caught at Each Station. Only
Stations at which Fish were Taken are Included

N.Z.O.I. Station No.	Date	Depth in Metres	Number of Fish	Position Lat.	Long.
A–448	10.1.59	752	3	77° 27′ S	172° 22′ E
A–449	11.1.59	362	5	77° 05′ S	177° 12′ E
A–453	13.1.59	2195–0	1*	75° 09′ S	171° 00′ W
A–454	14.1.59	914–828	1	73° 56′ S	176° 30′ W
A–456	15.1.59	238–201	4	74° 30′ S	179° 40′ W
A–457	16.1.59	315–342	6	75° 02′ S	175° 50′ E
A–459	16.1.59	534–549	32	75° 17′ S	172° 20′ E
A–460	17.1.59	415–430	22	75° 38′ S	168° 32′ E
A–461	18.1.59	578–567	7	73° 32′ S	171° 22′ E
A–466	24.1.59	569	7	78° 26′ S	174° 50′ W
A–467	26.1.59	88–183	1	77° 25′ S	169° 28′ E
A–468	26.1.59	110	2	76° 59′ S	167° 36′ E

*This fish was found in a vertical plankton haul (one metre net). Depth of capture
is unknown.

The number of fishes caught in the trawls appears to correlate roughly
with the size of trawl used. The smallest trawl, with a mouth measurement
of 7 in. by 20 in., caught only three fish in three hauls; the mid-size, with
a mouth of 10 in. by 32½ in., caught 20 fish in two tries; and the largest
aboard, 18 in. by 40 in., took 68 fish in four tries. These comparative
efficiencies suggest that gratifying results might be obtained from use of a
large trawl specially designed for fish.

Method of Preservation

Each fish was injected immediately upon capture. A solution of 10%
formalin from a 10 cc. hypodermic syringe was used to make three injec-
tions on each fish. The first was made into the heart or the conus arteriosus,
for the purpose of killing quickly before the stomach contents could be
regurgitated. The second injection was made to the stomach to stop digestive
action on the material stored there. The third injection was made to the
coelom for general visceral preservation. The fish was placed in a 7%
solution of formalin and allowed to harden for one to three days, and was
then wrapped in cheesecloth with the other fish from the station. This
bundle of fish was stored in five gallon cans in 5% formalin. For shipment
the cans were drained, and the fish left wrapped in the wet cheesecloth
within the cans, which were then sealed. These were hand-carried via
ship and plane to Long Beach State College where the fish were washed in
fresh water for twenty-four hours, or soaked until free of formalin odour,
then placed in 40% isopropyl alcohol.

Results

Ninety-one fish were obtained in bottom trawls at the twenty-one stations.
Successful trawls were made at 17 of these stations and fish were present in
trawls from eleven stations (Fig. 1). From the nature and quantity of the
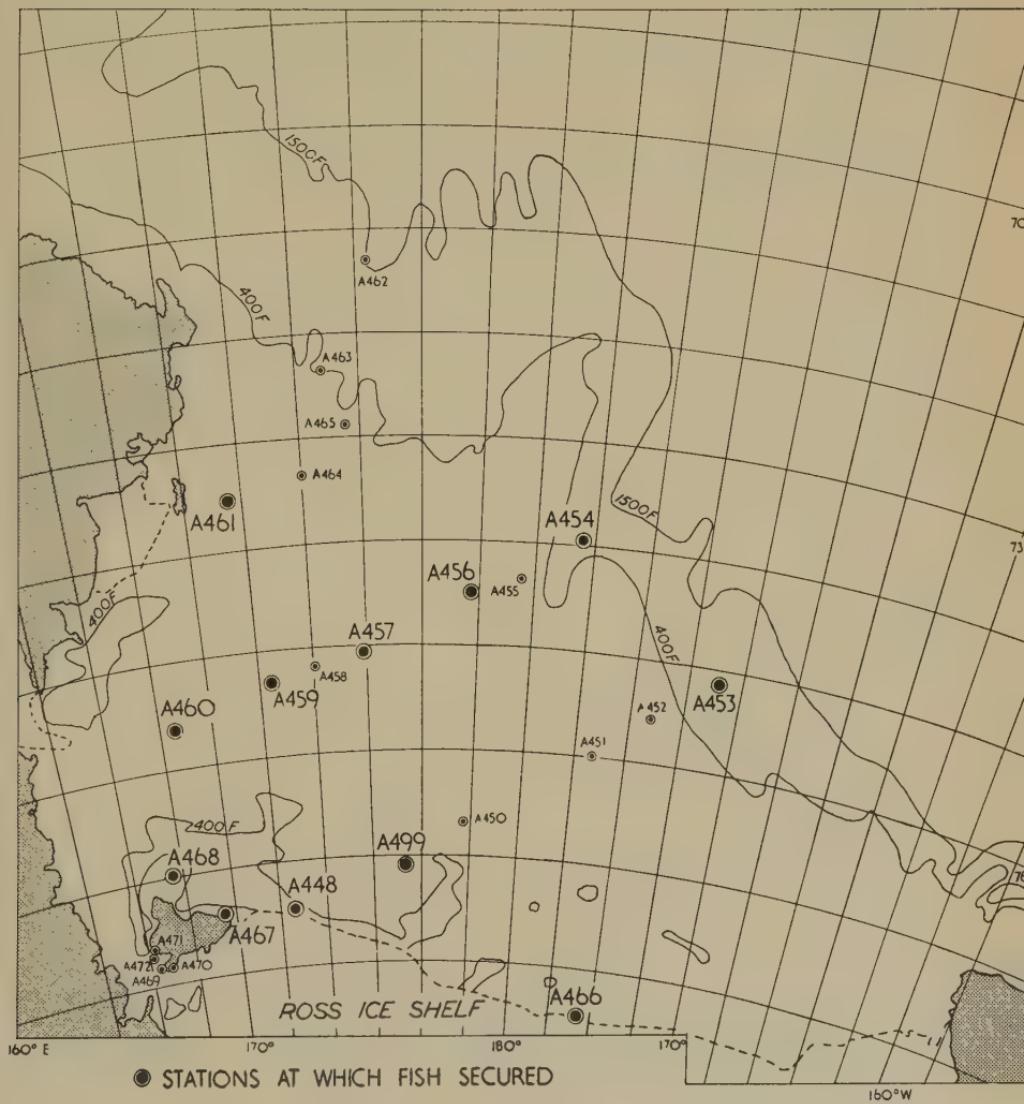
FIG. 1—Locations of stations from which fish were secured. (NOTE: Sta. A 499 should be Sta. A 449.)

benthic material secured, it seems highly probable that most fish secured were captured when near the bottom. The trawl bags were commonly half to three-quarters full and would not have fished efficiently after leaving the bottom in this condition.

One fish was captured in the vertical plankton net (Sta. A 453). There is no way of telling at what depth it was obtained. This makes a total of ninety-two fish.

The identifications of fish from each station are given in Table 2. To facilitate sorting, field numbers in an EN series were given each specimen as collected: these numbers appear in the table.

Table 2—Station List and Fish Species Obtained

(All lengths in station list are total lengths, as specimens were all complete. Total length is the length from tip of snout to tip of caudal fin.)

Station A–448
Date 10.1.59; Position 77° 27′ S, 172° 22′ E; Depth 752 metres;
Gear T.A.S.; Bottom mud.

Family Harpagiferidae

    Dolloidraco longedorsalis Roule
        3 spms.   52–98 mm   EN. 1, 2, 3.

Station A–449
Date 11.1.59; Position 77° 05′ S, 177° 12′ E; Depth 362 metres;
Gear T.A.S.; Bottom mud.

Family Nototheniidae

    Trematomus scotti (Boulenger)
        1 spm.   95 mm   EN. 5.
        1 spm. as yet unidentified   EN. 4.
    Pleuragramma antarcticum Boulenger
        1 spm.   121 mm   EN. 7.

Family Harpagiferidae

    Histiodraco vellifer (Regan)
        1 spm.   176 mm   EN.6.
    Artedidraco loennbergi Roule
        1 spm.   61 mm   EN. 8.

Station A–453
Date 13.1.59; Position 75° 09′ S, 171° 0′ W; Depth 2195 metres;
Gear Plankton net; Bottom yellow-brown mud.

Family Chaenichthyidae

    Pagetopsis macropterus (Boulenger)
        1 spm.   60 mm   EN. 9.

Station A–454
Date 14.1.59; Position 73° 56′ S, 176° 30′ W; Depth 914–828 metres;
Gear T.A.S.; Bottom rock.

Family Bathydraconidae

    Juvenile, as yet unidentified.
        1 spm. EN. 10.

TABLE 2—Station List, etc.—*continued*

Station A–456
Date 15.1.59; Position 74° 30′ S, 179° 40′ W; Depth 238–201 metres;
Gear T.A.S.; Bottom stones, gritty mud.

Family NOTOTHENIDAE

Trematomus lepidorhinus (Pappenheim)
    1 spm.   114 mm   EN. 11.
Trematomus scotti (Boulenger)
    3 spms.   70–85 mm   EN. 12, 13, 14.

Station A–457
Date 16.1.59; Position 75° 02′ S, 175° 50′ E; Depth 315–342 metres;
Gear T.P.; Bottom mud.

Family MURAENOLEPIDAE

Muraenolepis microps Lönnberg
    1 spm.   133 mm.   EN. 18.

Family NOTOTHENIDAE

Trematomus scotti (Boulenger)
    3 spms.   18–146 mm   EN. 15, 16, 17.

Family BATHYDRACONIDAE

Juveniles (one in poor condition), as yet unidentified
    2 spms.   68–75 mm   EN. 19, 20.

Station A–459
Date 16.1.59; Position 75° 17′ S, 172° 20′ E; Depth 534–549 metres;
Gear T.P.; Bottom soft mud.

Family NOTOTHENIDAE

Trematomus scotti (Boulenger)
    12 spms.   67–168 mm   EN. 22, 23, 25, 31, 34, 36, 37, 41, 45, 49, 50, 51,
Trematomus loennbergi Regan
    1 spm.   216 mm   EN.27.
Trematomus hansoni Boulenger
    1 spm.   136 mm   EN.33.
Trematomus sp.
    1 spm.   72 mm   EN. 47.
As yet unidentified
    1 spm.   83 mm   EN. 42.

Family HARPAGIFERIDAE

Dolloidraco longedorsalis Roule
    4 spms.   72–116 mm   EN. 24, 30, 43, 53.
Pogonphryne scotti Regan × *P. marmoratus** Norman
    2 spms.   108–203 mm   EN. 26, 46

Family BATHYDRACONIDAE

Racovitzia harrissoni (Waite)
    1 spm.   162 mm   EN. 48.
Bathydraco macrolepis Boulenger
    1 spm.   92 mm   EN. 44.
Bathydraco sp. as yet unidentified.
    1 spm.   80 mm   EN. 52.

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*Characteristics fall between those of *P. scotti* and *P. marmoratus*, but are closer to
P. scotti.

Table 2—Station List, etc.—*continued*

Family CHAENICHTHYIDAE

>   *Pagetopsis macropterus* (Boulenger)
>   2 spms.   164 mm   EN. 29, 35.
>   *Cryodraco antarcticus* Dollo
>   2 spms.   368–393 mm   EN. 32, 39.
>   *Chionodraco markhami* Miller and Reseck*
>   1 spm.   304 mm   EN. 40.

Family ZOARCIDAE

>   *Austrolycichthys concolor* (Roule and Despax)
>   2 spms.   209–214 mm   EN. 28, 38.

Station A–460
Date 17.1.59; Position 75° 38′ S, 168° 32′ E; Depth 415–430 metres;
Gear T.P.; Bottom gritty mud.

Family NOTOTHENIDAE

>   *Trematomus scotti* (Boulenger)
>   7 spms.   137–197 mm   EN. 56, 57, 58, 65, 71, 72, 73.
>   *Trematomus loennbergi* Regan
>   1 spm.   203 mm   EN. 67

Family HARPAGIFERIDAE

>   *Dolloidraco longedorsalis* Roule
>   2 spms.   82–100 mm   EN. 62, 69.
>   *Histiodraco vellifer* Regan
>   1 spm.   97 mm   EN. 61.
>   *Pogonophryne scotti* Regan
>   1 spm.   267 mm   EN. 66.

Family BATHYDRACONIDAE

>   *Racovitzia harrisoni* (Waite)
>   1 spm.   278 mm   EN. 59.
>   *Bathydraco nudiceps* Waite
>   2 spms.   130–133 mm   EN. 54, 55.
>   *Gerlachea australis* Dollo
>   1 spm.   236 mm   EN. 60
>   As yet unidentified
>   1 spm.   98 mm   EN. 63.

Family CHAENICHTHYIDAE

>   *Chionodraco kathleenae* Regan
>   2 spms.   216–310 mm   EN. 64, 68.
>   *Chionodraco markhami* Miller and Reseck*
>   1 spm.   325 mm   EN. 70.
>   *Pagetopsis macropterus* (Boulenger)
>   2 spms.   117–127 mm   EN. 74, 75.

Station A–461
Date 18.1.59; Position 73° 32′ S, 171° 22′ E; Depth 578–567 metres;
Gear T.P.; Bottom sandy mud.

Family NOTOTHENIDAE

>   *Trematomus scotti* (Boulenger)
>   2 spms.   155–156 mm   EN. 76, 77.
>   *Pleuragramma antarcticum* Boulenger
>   1 spm.   143 mm   EN. 78.

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*Manuscript name. A description of this fish is in press (Miller and Reseck).

TABLE 2—Station List, etc.—*continued*

Family HARPAGIFERIDAE
 Artedidraco loennbergi Roule
  1 spm. 94 mm EN. 79.
 Dolloidraco longedorsalis Roule
  1 spm. 84 mm. EN. 80.
 Pogonphryne scotti Regan × *P. marmoratus* Norman
  1 spm. 102 mm EN. 82.
Family CHAENICHTHYIDAE
 Pagetopsis macropterus (Boulenger)
  1 spm. 115 mm EN. 81.

Station A–466
Date 24.1.59; Position 78° 26′ S, 174° 50′ W; Depth 569 metres;
Gear T.A.S.; Bottom mud.
Family NOTOTHENIDAE
 Trematomus scotti (Boulenger)
  1 spm. 96 mm EN. 87.
 Trematomus loennbergi Regan
  1 spm. 298 mm EN. 88.
Family HARPAGIFERIDAE
 Dolloidraco longedorsalis Roule
  1 spm. 118 mm EN. 84.
Family BATHYDRACONIDAE
 Bathydraco nudiceps Waite
  4 spms. 90–136 mm EN. 83, 85, 86, 89.

Station A–467
Date 26.1.59; Position 77° 25′ S, 169° 28′ E; Depth 88–183 metres;
Gear, D.N.; Bottom rocks.
Family NOTOTHENIDAE
 Trematomus scotti (Boulenger)
  1 spm. 108 mm. EN. 90.

Station A–468
Date 26.1.59; Position 76° 59′ S, 167° 36′ E; Depth 110 metres;
Gear T.A.S.; Bottom not known.
Family NOTOTHENIDAE
 Trematomus centronotus Regan
  1 spm. 187 mm EN. 91.

Family BATHYDRACONIDAE
 Juvenile as yet unidentified.
  EN. 92.

A systematic list of the fishes is given in Table 3. A number of the fishes collected have not at the time of writing been identified completely. Of ninety-two fish (including one specimen, EN. 68/A1, from a fish stomach), seventy-eight have been assigned to species; four to genus only, and four to family only: six are unnamed.

Of the field numbers allocated, ten do not appear in the systematic list of Table 3. The fish, EN. 10, 19, 20, 52, 63, and 92, are juvenile or other Bathydraconidae not identified; EN. 4, and 42 are of the Notothenidae, and N68/A1, a stomach specimen, is of the Chaenichthyidae: the field number EN. 21 was not used.

Sig. 8

Table 3—Systematic List of Ross Sea Fishes from the *Endeavour* Cruise of 1959.
Determinations by Richard Gordon Miller

Family
  MURAENOLEPIDAE
    Muraenolepis microps Lönnberg   1 spm.   133 mm,*   Sta.   A   457.
                                            EN. 18.

Family
  NOTOTHENIDAE
    Trematomus hansoni Boulenger   1 spm. 115 mm, Sta. A 459, EN. 33.
    Trematomus loennbergii Regan   3 spms. 172–234 mm, Sta. A 459,
                                            A 460, A466. EN. 27, 67, 88.
    Trematomus centronotus Regan   1 spm. 160 mm, Sta. A 468. EN. 91.
    Trematomus scotti (Boulenger)   30 spms. 55–143 mm, Sta. A 449–
                                            467. EN. 5, 12, 13, 14, 15, 16, 17,
                                            22, 23, 25, 31, 34, 36, 37, 41, 45,
                                            49, 50, 51, 56, 57, 58, 65, 71, 72,
                                            73, 76, 77, 87, 90.
    Trematomus lepidorhinus (Pappenheim) 1 spm. 98 mm, Sta. A 456. EN. 11.
    Trematomus sp.   1 spm. 71 mm, Sta. A 459. EN. 47
    Pleuragramma antarticum Boulenger   2 spms. 121, 143 mm, Sta. 4 449,
                                            A 461. EN. 7, 78.

Family
  HARPAGIFERIDAE
    Artedidraco loennbergi Roule   2 spms. 48, 76 mm, Sta. A 449,
                                            A 461. EN. 8, 79.
    Dolloidraco longendorsalis Roule   11 spms. 38–118 mm, Sta. A 448,
                                            A 459, A 460, A 461, A 466.
                                            EN. 1, 2, 3, 24, 30, 43, 53, 62, 69,
                                            80, 84.
    Histiodraco velifer Regan   2 spms. 76, 137 mm, Sta. A 449,
                                            A 460. EN. 6, 61.
    Pogonophryne scotti Regan   1 spm. 267 mm.† Sta. A. 460.
      (close to *P. marmoratus* Norman)   EN. 66.
    Pogonophryne sp.   3 spms. 80–160 mm, Sta. A 459,
      (between *P. scotti* Regan and *P. mar-* A 461. EN. 26, 46, 82.
      moratus Norman)

Family
  BATHYDRACONIDAE
    Bathydraco macrolepis Boulenger   1 spm. 82 mm, Sta. A 459. EN. 44.
    Bathydraco nudiceps Waite   6 spms. 76–120 mm, Sta. A 460,
                                            A 466. EN. 54, 55, 83, 85, 86, 89.
    Gerlachea australis Dollo   1 spm. 131 mm, Sta. A 460. EN. 60.
    Racovitzia harrisoni (Waite)   2 spms. 162, 278 mm, Sta. A 459,
                                            A 460. EN. 48, 59.

Family
  CHAENICHTHYIDAE
    Pagetopsis macropterus (Boulenger)   6 spms. 54–153 mm, Sta. A 451,
                                            A 453, A 459, A 460, A 461.
                                            EN. 9, 29, 35, 74, 75, 81.
    Chionodraco kathleenae Regan   2 spms. 265, 266 mm, Sta. A 459,
                                            A 460. EN. 64, 68.
    Chionodraco markhami Miller and Reseck 2 spms. 273 mm (paratype) 295 mm
                                            (holotype), Sta. A 460. EN. 40, 70.
    Cryodraco antarcticus Dollo   2 spms. 368, 393 mm, Sta. A 459.
                                            EN. 32, 39.

Family
  ZOARCIDAE
    Austrolycichthys concolor (Roule and 2 spms. 209, 214 mm,† Sta. A. 459.
      Despax)   EN. 28, 38.

*Standard lengths are given throughout the list (length from tip of snout to posterior
 end of caudal peduncle), except where designated otherwise.
†In total length.

ACKNOWLEDGMENTS

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INFLUENCE OF TUSSOCK GRASSES ON ZONATION
OF ACCOMPANYING SMALLER SPECIES

By D. SCOTT, Botany Department,* University of Otago.

Summary

Part 1. The nearest neighbour method is extended to the study of association between distributions of different species.

Part 2. The nearest neighbour method is suitable for detecting association between the distribution of tussock grassland species.

Tussocks influence the zonation of accompanying smaller species. There is both a general aggregation towards the tussock base, and a species difference in zonation with respect to the tussocks, in that each species tends to occupy a particular zone around the tussock.

PART 1: EXTENSION OF "NEAREST NEIGHBOUR" METHODS
TO INTERSPECIFIC RELATIONSHIPS

INTRODUCTION

In applying the "nearest neighbour" sampling techniques to a single species population, the distances measured are from a randomly chosen plant to its nearest neighbour; or between a randomly chosen point and the nearest plant (Clark and Evans 1954; Cottom and Curtis 1956; Greig-Smith 1957; Moore 1954; Skellam 1952). The density can then be estimated from the frequency distribution of distances, if it is assumed that the individuals are randomly distributed. It is for determining density that the method is usually used.

Alternatively if the density dependent factor can be determined by an independent means, or be eliminated from the theoretical distribution, then a comparison of the actual and expected distributions provides a test of randomness in the population (e.g. Pielou 1959). This alternative is used to extend the nearest neighbour technique to the study of interspecific relationships.

Expected Distributions for Species Populations

This section is by H. Silverstone, Mathematics Department, University of Otago, and is gratefully acknowledged.

A and B are two sets of points in a plane, the members of A being randomly dispersed among themselves, and the members of B being ran-

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domly situated with respect to the members of A. Given any point b of B the probability differential of the distance x to the nearest point of A is

$$dP = \frac{2}{\phi} x \exp\left(-\frac{x^2}{\phi}\right) dx \qquad \text{...................(1)}$$

where

$$\phi = \frac{1}{\pi D}$$

and D is the number of members of A per unit area.

From this it follows that $\dfrac{2x^2}{\phi}$ is distributed as Chi-square for 2 degrees of freedom.

Given n independent points of B and the respective distances x_i $(i = 1, 2, ---, n)$ to the nearest members of A, the quantity

$$K = \frac{2 \, \Sigma \, x^2}{\phi} \qquad \text{...................(2)}$$

is distributed as Chi-square for $2n$ degrees of freedom.

If for two independent sets B_1 and B_2, each randomly situated with respect to A, we have

$$K_1 = \frac{2 \, \Sigma_1 x^2}{\phi} \qquad \text{...................(3)}$$

$$K_2 = \frac{2 \, \Sigma_2 x^2}{\phi}$$

then $\dfrac{K_1/n_1}{K_2/n_2}$ is distributed as F for $2n_1$, $2n_2$ degrees of freedom. That is,

$$F = \frac{\Sigma_1 \dfrac{x^2}{n_1}}{\Sigma_2 \dfrac{x^2}{n_2}} \qquad \text{...................(4)}$$

or the ratio of the mean square distances is distributed as F for $2n_1$, $2n_2$ degrees of freedom. (The appropriate test is a two-tailed test.)

If ϕ is known, then the Chi-square distribution may be used to test the hypothesis that the members of B are randomly situated with respect to those of A.

Suppose ϕ is not known but we know K_0 the mean square distance of a set n_0 of points of A from their nearest neighbours in A. Then since the probability differential for the distance of a point of A from its nearest neighbour in A is the same as in (1) above, it follows that we may use

$$F = \frac{K_0/n_0}{K_1/n_1} = \frac{\Sigma_0 \dfrac{x^2}{n_0}}{\Sigma_1 \dfrac{x^2}{n_1}} \qquad \text{...............(5)}$$

to test the hypothesis that B is randomly situated with respect to A.

For two independent "satellite" populations B_1 and B_2, the quantity F given in (4) will serve to test the hypothesis that both populations are randomly situated with respect to the "host" population A, but not to discriminate in favour of any particular alternative hypothesis.

However, from (5) above it is clear that the degree of dependence of a "satellite" on the "host" varies inversely as the mean square distance of the satellite from the host. Hence, if we rank a set of satellites B_1, B_2 – – – – in increasing order of magnitude of their mean square distances from A we are ipso facto ranking them in order of dependence on A.

The choice of the "host" species to which other measurements are made will depend on independent considerations as to which species has the major influence in a community.

PART 2: APPLICATION OF METHOD TO TUSSOCK GRASSLAND

FIELD PROCEDURE

The area studied was short tussock grassland dominated by *Festuca novae-zealandiae* in the Godley Valley, Lake Tekapo (Scott 1959).

In what appeared to be a uniform area a strip 10 ft wide and of indefinite length was selected. A start was made at one end and small areas were chosen that had tussocks of about average height and spread. In these the distance from the smaller species to the nearest tussock were measured. The distance measured was from $\frac{1}{2}$ in. (approx.) within the tussock clump to the point where the subsidiary plant appeared to have first become established. Neither rare species nor those which have a mat forming habit are amenable to this kind of measurement so that results are given for only 20 of the 40 or so species that were present.

In selecting the plants to be measured a compromise had to be made between the random sampling demanded by theory and what is practically possible in the field. Within species the plants were sampled in batches of about ten. Measurements were made from the first ten plants encountered of a particular species and another batch was not measured until there were groups of similar measurements for each of the other species. Sampling in

batches partly ensured measurements of both abundant and scarce species throughout the strip investigated. The observations were confined to a strip to prevent the tendency to follow local denser areas of particular species.

<div align="center">RESULTS</div>

These are given for two areas:

> Area A: Short tussock on a hill (approx. 34°). Moderate grazing had resulted in a general clipped appearance.
> Area B: Short tussock on flat. Growth is more diffuse through light grazing. In area A the unit used was 1 in. and area B, ½ in.

(a) Accuracy of the distance measurement

The suitability of the nearest neighbour technique depends on the plants occurring as discrete and readily distinguishable individuals. This is not a feature of tussock grasslands.

An estimate of the error in measurements was obtained by repeating measurements on a set of marked plants. In 100 plants the mean difference in repeated measurements was 0·29 in. This on the average is about 5%–10% of the total distance measured.

(b) Comparison of the aggregate distribution for all species and the fitted expected distribution.

This indicates whether the smaller plants, irrespective of species, are randomly distributed with respect to the tussock plants. The value of ϕ was estimated from the data and the expected distribution calculated using the null hypothesis that the individuals were randomly dispersed.
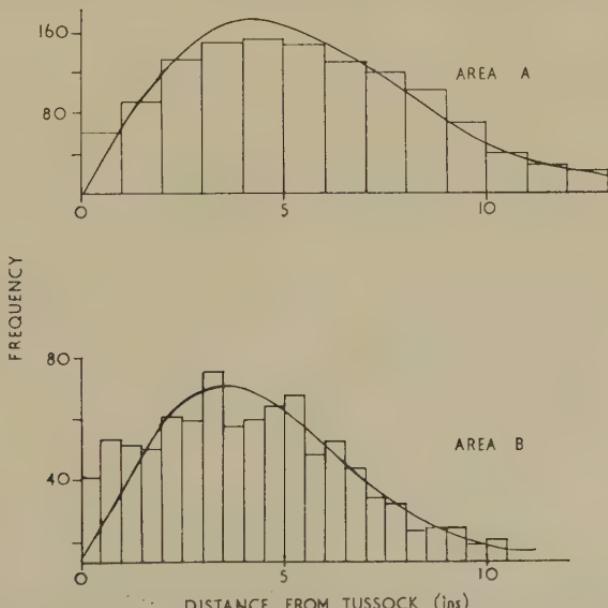
Fig. 1—Comparison of observed and expected distribution for plants of all species. The expected distribution is drawn as a smooth curve joining the calculated expected frequencies plotted at the centres of their class intervals.

The Chi-square tests of Goodness-of-Fit gave values of $39 \cdot 0 (11 d.f.)$ and $156 (19 d.f.)$ for areas A and B respectively. Since both these values are highly significant the null hypothesis is rejected. It is clear from Fig. 1 that the main significant departures from expectation were caused by the high observed frequencies in the first class(es) in each case. These are species aggregated very near or within the tussock and must reflect some general favourable influence of the tussock.

(c) The relative distributions of species with respect to the tussocks.

In Table 1 the results for individual species have been arranged in order of magnitude of K. As established in Part 1 this also ranks them in order of dependence on the tussock plant.

The F test established in Part 1 may be used to test whether pairs of species are *both* randomly distributed. But the F test cannot be used to discriminate degrees of dependence on the tussock for if the species are non-random – as the results suggest – then the F test is not longer applicable. This finer discrimination must await knowledge of the actual distribution of the species relative to the tussock.

There is a general correspondence in order for species common to both areas e.g. *Wahlenbergia* and *Hydrocotyle*. Also there is no marked difference between indigenous and exotic species.

DISCUSSION AND CONCLUSIONS

The nearest-neighbour technique can be very useful for detecting associations in small areas of a plant community, particularly where there is a contrast in plant sizes as in a tussock grassland.

The results support a hypothesis that the tussocks do influence the zonation of associated plants and that different species vary in their response, i.e., each species tends to occupy a particular zone around the tussock.

Probably it is the favourable influence of the tussock plants on the microclimate in their immediate vicinity that is the cause of this partial zonation of associated smaller species. Other factors contributing to this could be the protection from grazing near the tussock base, or root competition between the various species. These hypotheses require investigation.

ACKNOWLEDGMENTS

TABLE 1—Mean and Mean Square Distance from Tussock to Smaller Associated Species

AREA A*				AREA B			
Species	n	$K = \dfrac{\Sigma x^2}{n}$	Mean Distance (in.)	Species	n	$K = \dfrac{\Sigma x^2}{n}$	Mean Distance (in.)
Acaena anserinaefolia	50	12.9	2.7	Viola cunninghamii	51	6.4	1.8
Senecio lagopus	49	20.0	3.8	Wahlenbergia albomarginata	51	15.2	3.2
Wahlenbergia albomarginata	54	23.4	3.8	Geranium sessiliflorum	17	15.2	3.4
Celmisia gracilenta	51	25.7	4.3	Senecio lagopus	51	18.1	3.7
Ranunculus lappaceus	70	27.3	4.6	Leucopogon fraseri	50	20.3	4.0
Leucopogon fraseri	70	31.6	4.9	†Crepis capillaris	50	20.6	3.9
†Rumex acetosella	64	31.7	5.0	Celmisia gracilenta	50	20.9	4.0
Helichrysum filicaule	63	31.7	4.5	Cotula perpusilla	51	21.6	4.4
Luzula campestris	70	36.5	5.2	Stackhousia minima	50	24.7	4.6
†Cerastium caespitosum	59	36.8	5.5	†Linum catharticum	54	25.7	4.6
†Crepis capillaris	70	41.2	5.9	†Rumex acetosella	52	26.7	4.7
Geranium sessiliflorum	60	41.6	5.8	†Hypochaeris radicata	52	27.2	4.6
†Anthoxanthum odoratum	70	42.6	5.9	Helichrysum filicaule	50	26.2	4.6
Epilobium chloraefolium or eru-bescens	70	46.2	6.5	Luzula campestris	50	27.5	4.8
†Hypochaeris radicata	70	51.9	6.7	Lagenophora cuneata	50	28.5	4.8
†Centaurium umbellatum	60	51.5	6.7	†Anthoxanthum odoratum	53	31.5	4.9
Hydrocotyle novae-zelandiae	46	52.4	6.7	Pimelea prostrata	52	32.2	5.0
Oreomyrrhis andicola	70	52.5	6.6	Colobanthus strictus	30	32.7	5.4
Gnaphalium collinum	70	64.7	7.2	Pterostylis mutica	50	33.0	5.0
Indigenous	793	36.8		Hydrocotyle novae-zelandiae	54	34.7	5.2
Exotic	393	42.7		Epilobium chloraefolium or eru-bescens	51	39.2	6.6
				Indigenous	758	25.0	
				Exotic	261	26.2	

*Viola cunningbamii present but not included in Table.   †Exotic species.

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A REVISED CLASSIFICATION OF THE ORDER ENOPLIDA (NEMATODA)

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Summary

The order Enoplida is re-classified into seven sub-orders mainly on the basis of the arrangement of the oesophageal glands and their ducts. The sub-orders recognised are Enoplina, Alaimina **n. sub-order,** Dorylaimina, Trichosyringina (composed of the Mermithoidea and Trichuroidea) and Dioctophymatina. The Mononchidae are removed from the Tripyloidea and with the Bathyodontidae **n. fam.** (type genus *Bathyodontus* Fielding, 1950) comprise the Mononchoidea **n. superfamily** within the Dorylaimina. The Belondiridae and Nygolaimidae have been redefined, *Nygellus* Thorne, 1939 is transferred from the former to the latter and the Nygolaimellinae **n. subfam.** (type genus *Nygelolaimellus* Loos, 1949) erected. The Campydorinae are removed from the Leptonchidae and elevated to family rank. The Trichodorinae are given family rank, and with the Diphtherophoridae form the Diphtherophoroidea within the Dorylaimina. In the Trichuroidea four families and four subfamilies are recognised (Table 1). Skrjabin & Schikhobalova's 1954 proposal to unite the Trichuroidea with the Dioctophymatina is rejected on the grounds of morphology and life history. It is considered that the life history of *Dioctophyme renale* indicates a relationship between the Dioctophymatina and the Gordiacea. The name *Octonchus* **nom. nov.** is proposed pro *Polydontus* Schultz, 1932 preocc., nec. *Polydontus* Blainville, 1826 a mollusc.

INTRODUCTION

Filipjev (1934) and Chitwood (1937) have rendered a signal service to nematology in leading the way towards a satisfactory classification of the Nematoda as a whole. Chitwood's (1937) classification was reprinted in expanded form in Chitwood and Chitwood's (1937) *"Introduction to Nematology",* and again with some modifications in the revised edition of 1950. T. Goodey (1951) in *"Soil and Freshwater Nematodes"* used the Chitwoods' 1937 classification for the most part, with modifications from Thorne (1939) in the Dorylaimoidea. The later works of the Chitwoods, T. Goodey, and Thorne have firmly established the main points of Chitwood's 1937 classification of the Enoplida for workers on soil and plant parasitic nematodes. Workers on marine nematodes use a slightly different classification; e.g. Wieser (1953a) regards all the subfamilies of the Enoplidae as families. The taxonomy of the Enoploidea needs extensive study; that proposed here will suffice in the interim. Because of the small number of animal parasitic genera contained within the Trichuroidea and the Dioctophymatina animal parasitologists have, with the exception of Russian workers, been little concerned of late with the higher classification of this order. The exception is Skrjabin & Schikhobalova in Skrjabin, Schikhobalova, Sobolev, Paramonov & Svaarikov (1954) who have proposed a number of changes in the classification of these forms. The suggested changes include the removal of the Trichocephalata (suborder) Skrjabin &

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Shultz, 1928 (= Trichuroidea Railliet, 1916) from the Enoplida and uniting them with Dioctophymata Skrjabin, 1927 (suborder) under the name Trichocephalida Spasski, 1954 in Skrjabin *et al.,* 1954. No reasons are given for these changes.

If the Trichuroidea are to be united with any other group at the ordinal or subordinal level their logical partners are the Mermithoidea with which they share many features, e.g. the oesophageal glands are represented by a stichosome outside the contour of the oesophagus, pore-like amphids, often a single spicule, and the presence of a stylet in the larval stage which, as Fülleborn (1923) pointed out, suggests an affinity between the two groups. The stylet occasionally persists in adults of both superfamilies. The Dioctophymatina on the other hand are characterised by the presence of highly polynucleate oesophageal glands, which discharge anteriorly, contained within the contour of the oesophagus, and a conspicuous caudal sucker in the males – all features not found in the Trichuroidea. Woodhead (1950) has confirmed the observation of Lukasiak (1930) that the first stage larva of *Dioctophyme renale* possesses a stylet. This may be regarded as indicative of an affinity between the dioctophymatids, the trichurids, mermithids, and the dorylaimids, and would justify the retention of all these groups within the Enoplida. Primarily because of the differences in the arrangement of the oesophageal glands, and because of the occurrence of a *Gordius*-like stage in the life history I prefer to keep the Dioctophymatina separate from the Trichuroidea, though admitting a degree of affinity on other grounds as suggested by Rauther (1918).

The great similarity of life history and of the morphology of larval stages of *Dioctophyme renale* (Woodhead, 1950) and gordiids appears to indicate that these two groups may be much closer phylogenetically than has hitherto been supposed.

The presence of a dorylaimoid oesophagus in the Tetradonematidae, a dorylaimoid spear in all mermithid larvae, and of cyathiform amphids in some, as well as the pre-anal supplements in males point as Steiner (1917) and Filipjev (1934) have suggested, to an affinity between the Mermithidae and the Dorylaimoidea. In the work which follows Skrjabin *et al's* proposals are rejected.

Hyman (1951) gave a classification of the Nematoda into 17 orders without higher groups. The diagnoses are vague and insufficiently precise to be useable. The Chitwoods' (1937) division into two main groups Phasmidia or Sercenentea, and Aphasmidia or Adenophorea is retained as a matter of taxonomic expediency. The usefulness of these divisions in practical nematology is doubtful.

The present study arose out of the writer's work on the Mononchidae (Clark, 1960 a, b, c 1961 a, b c) during the course of which it became apparent that the Mononchidae had very little in common with the rest of Chitwood's Tripyloidea, but much in common with the Dorylaimoidea. As it is now over twenty years since Chitwood's classification was erected and as much new knowledge has become available in the meantime the opportunity is taken to incorporate this into the existing framework and to make such changes as seem necessary.

TABLE 1—Proposed Classification of the Enoplida

Order ENOPLIDA

Suborder	Superfamily	Family	Subfamily
ENOPLINA	ENOPLOIDEA	ENOPLIDAE	Enoplinae Leptosomatinae Phanodermatinae Oxystominae
		LAURATONEMATIDAE	
		ONCHOLAIMIDAE	Oncholaiminae Eurystominae Enchilidiinae
	TRIPYLOIDEA	TRIPYLIDAE	
		IRONIDAE	Ironinae Cryptonchinae
ALAIMINA		ALAIMIDAE	
DORYLAIMINA	MONONCHOIDEA	MONONCHIDAE BATHYODONTIDAE	
	DORYLAIMOIDEA	DORYLAIMIDAE	Dorylaiminae Actinolaiminae Tylencholaiminae
		OPAILAIMIDAE BELONDIRIDAE	
		NYGOLAIMIDAE	Nygolaiminae Nygolaimellinae
		LEPTONCHIDAE CAMPYDORIDAE	
	DIPHTHEROPHOROIDEA	DIPHTHEROPHORIDAE TRICHODORIDAE	
TRICHOSYRIN-GINA	MERMITHOIDEA	MERMITHIDAE TETRADONEMATIDAE	
	TRICHUROIDEA	TRICHURIDAE	Trichurinae Capillariinae
		TRICHOSOMOIDIDAE	Trichosomoidinae Anatrichosomatinae
		TRICHINELLIDAE CYSTOOPSIDAE	
DIOCTOPHY-MATINA		DIOCTOPHYMATIDAE	Dioctophymatinae Eustrongylidinae
		SOBOLIPHYMATIDAE	

Proposed Classification

Chitwood's classification into suborders is based in part on the nature of the oesophageal glands and their ducts. It is proposed to utilise this character more fully in the subdivision of the Enoplida. On this basis it is possible to divide the order into five readily definable suborders (see Table 1 and Fig. 1). The type and arrangement of the oesophageal glands and their ducts in the different suborders is shown schematically in Fig. 1. In the Enoplina the oesophageal glands are uni- or multi-nucleate and at least three of the glands discharge into the oesophageal lumen anterior to the nerve ring, commonly in the stomatal region. In the Alaimina n. suborder there appears to be seven uninucleate oesophageal glands which discharge anterior to the nerve ring. The Dorylaimina are characterised by having five (only three in *Leptonchus*) usually uninucleate oesophageal glands with all the ducts posterior to the nerve ring. In the Trichosyringina the oesophageal glands lie outside the contour of the oesophagus as a stichosome. Highly polynucleate, dichotomously branching oesophageal glands which lie within the contour of the oesophagus and which discharge anteriorly to the nerve ring characterise the Dioctophymatina.
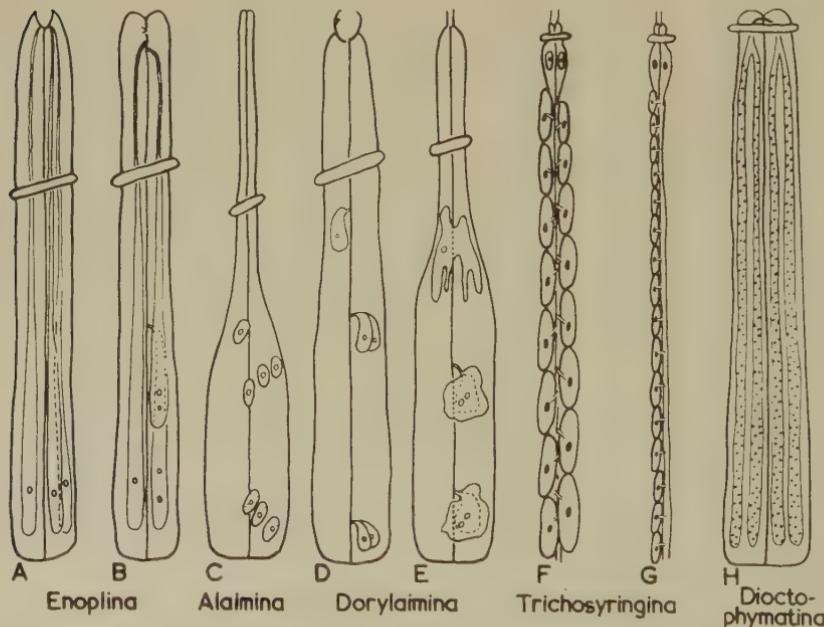
Fig. 1—Diagrams illustrating the various arrangements of the oesophageal glands and their ducts in the Enoplida. A. Enoploidea, B. Tripyloidea, C. Alaimidae, D. Mononchoidea, E. Dorylaimoidea, F. Cystoopsidae, G. Mermithoidea and Trichuroidea excluding Cystoopsidae, H. Dioctophymatina. (Partly after Chitwood & Chitwood.)
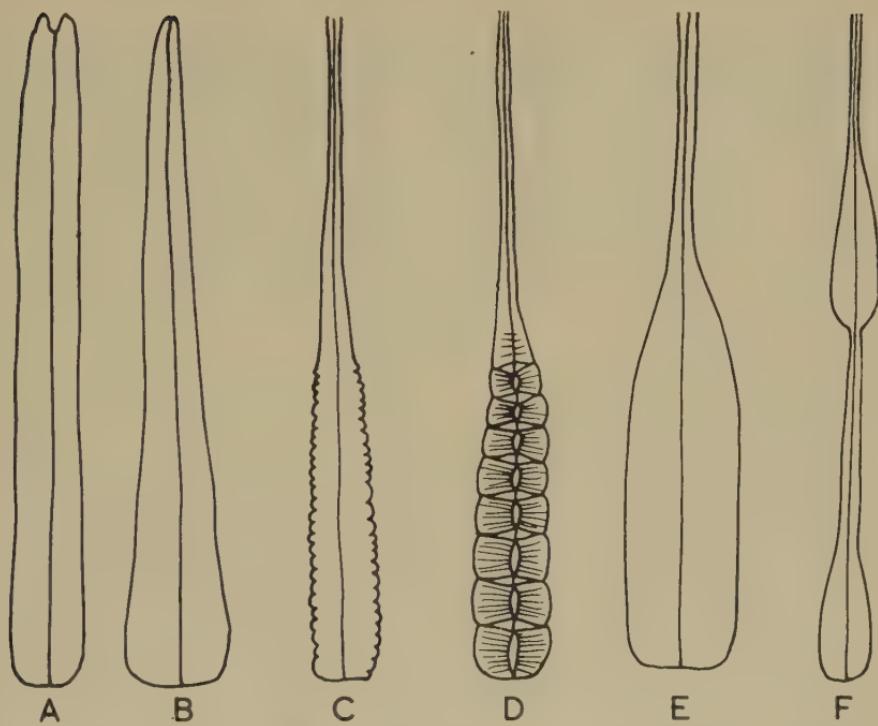
FIG. 2—Diagrams to illustrate the terminology used to describe types of oesophagus.
A. cylindroid, B. conoid, C. crenate, D. multibulbar, E. dorylaimoid, F. bi-bulbar.

At the level of superfamilies fewer changes have been made. The conception of the Tripyloidea has altered somewhat since 1937. Thorne (1939) removed the Alaimidae to the Dorylaimoidea. In 1950 the Chitwoods moved the Ironidae from the Enopliodea to the Tripyloidea, but retained the Alaimidae in the Tripyloidea. In the present scheme the Alaimidae are transferred to a newly created suborder Alaimina. The Alaimidae did not conform with the other members of the Tripyloidea, nor with other members of the Dorylaimoidea. The Alaimina possess a number of dorylaimoid and tripyloid characters as well as some special ones of their own. These characters are mentioned in Table 2. The greatly reduced stoma of the Alaimids is reminiscent of some Ironidae and male Enchilidiinae.

On balance it seems that the Alaimina may have an ancestry with much in common with the Tripyloidea and Dorylaimoidea, but have diverged along a different evolutionary line.

The Mononchidae have also been removed from the Tripyloidea, and in combination with the Bathyodontidae n. fam. form the Mononchoidea within the Dorylaimina. The Mononchidae have very little in common with the Tripyloidea, but share the following features with the Dorylaimoidea: similar arrangement of the oesophageal gland cells and their ducts; the absence of cephalic setae; a greatly reduced excretory system; the common occurrence of a sphincter muscle and glandular region at the junction of the

oviduct and uterus (T. Goodey, 1942, J. B. Goodey, Peacock and Pitcher 1960, Clark 1961 b, c); the large number of preanal supplements; the presence of cuticular caudal pores, and the occupation of the same general type of habitat.

Table 2—The Distribution of Certain Characters in the Alaimina, Dorylaimoidea, and Tripyloidea

Character	Alaimina	Dorylaimoidea	Tripyloidea
Stoma unarmed, reduced	+	—	—
seven oesphageal glands	+	—	—
testis single	+	—	—
oesophagus dorylaimoid	+	+	—
pair of adanal supplements present	—	+	—
prerectum present	—	+	—
spicules tripyloid	+	—	+
oesophageal gland ducts ant. to nerve ring	+	—	+
gubernaculum present	—	—	+
caudal glands present	—	—	+
cephalic setae present	—	—	+

The Dorylaimoidea have been further restricted to exclude the Diphtherophoridae (*sensu* Thorne, 1939) which have been raised to superfamily rank. The presence of the Diphtherophidae within the Dorylaimoidea has long been a matter of doubt, and because they differ from the rest of the Dorylaimoidea in so many characters e.g. a different type of spear, the presence of a gubernaculum, a distinct excretory pore, an unpaired testis, and the absence of a prerectum and adanal supplements it is considered advisable to remove them from the Dorylaimoidea. This action emphasises their distinctive characters and makes the Dorylaimoidea a more compact group. The Diphtherophoroidea may have to be removed from the Dorylaimina in the future.

In the following section the changes which have been made in the families, subfamilies and the distribution of genera and species are noted after the diagnoses of the relevant groups.

Diagnoses

In the diagnoses information given in the higher categories is not mentioned again in the lower ones except to restrict the diagnoses of the lower category. In the lists of genera synonyms are not new unless marked as such.

Order ENOPLIDA

Adenophorea.   Amphids pocket-like to pore-like or tuboid; caudal glands present or absent; subventral oesophageal glands opening either near anterior end of oesophagus or in posterior half; glands sometimes duplicate, rarely polynucleate; or outside the contour of the oesophagus as a stichosome; oesophagus cylindroid or conoid, rarely bi- or muti-bulbar, commonly with a narrow anterior part and a wider glandular posterior part, or greatly attenuated. Setae present or absent. Habitat – marine, freshwater, soil, parasitic in arthropods and vertebrates.

Suborder ENOPLINA

Enoplida.   Head usually bearing 6 + 10, 10, or 6 + 4 setae (setae absent in a few scattered genera); amphids usually pocket-like; typical stylet absent; three or more oesophageal glands opening in the stomatal region, glands mostly uninucleate; oesophagus grossly cylindrical, conoid, or multibulbar; intestine functional; ovaries reflexed, female reproductive system simple; male with two spicules (very rarely none or one); muscular caudal sucker absent; caudal glands usually present; hypodermal glands commonly well developed; excretory system absent or consisting of one ventral cell. Polymyarian or meromyarian. Habitat – mainly marine or freshwater, some genera in moist soil.

Superfamily ENOPLOIDEA Schuur.-Stekh. & de Coninck, 1933

Enoplina.   Cuticle of head duplicate; males with 0, 1 or 2 supplements.

Family ENOPLIDAE Baird, 1853

Enoploidea.   Stomatorhabdions poorly sclerotised; with or without mandibles; without a distinct stomatal capsule; stoma surrounded by oesophageal tissue. Type genus: *Enoplus* Dujardin, 1845.

Subfamily ENOPLINAE Micoletzky, 1922

Enoplidae.   With three bifurcate mandibles; oesophagus cylindrical; amphids pocket like; males with one tuboid preanal supplement. Type genus: *Enoplus* Dujardin, 1845.

Other genera—

Enoploides Savaljav, 1912
Enoplolaimus de Man, 1893
Enoplonema Kreis, 1934
Epacanthion Wieser, 1953
Hyalacanthion Wieser, 1959
Mesacanthion Filipjev, 1927
Mesacanthoides Wieser, 1953
Metenoploides Wieser, 1953
Oxyonchus Filipjev, 1927

Paramesacanthion Wieser, 1953
Parasavaljevia Wieser, 1953
Parenoplus Filipjev, 1927
Rhabdodemania Bayliss & Daubney, 1926
Savaljevia Filipjev, 1927
Trichenoplus Mawson, 1956
n. syn. *Filenoplus* Mawson 1956

Sig. 9

Genera doubtfully included—
Chaetonema Filipjev, 1927   *Fenestrolaimus* Filipjev, 1927
Donsinema Allgén, 1949

Subfamily Leptsomatinae Micoletzky, 1922

Enoplidae. Without mandibles; posterior part of oesophagus distinctly muscular; oesophagus usually cylindrical, rarely conoid, never vesiculate; subventral oesophageal glands may be multinucleate. Amphids pocket-like; males with one or two tuboid supplements (rarely absent, *Stenolaimus*). Type genus: *Leptosomatum* Bastian, 1865.

Other genera—

Anticoma Bastian, 1865
Anticomopsis Micol. & Kreis, 1930
Antopus Cobb, 1913
Barbonema Filipjev, 1927
Cylicolaimus de Man, 1890
(?syn. *Nudolaimus* Allgén, 1929)
Jägerskiöldia Filipjev, 1916
Leptosomatides Filipjev, 1918
Leptosomatina Allgén, 1951
Leptosomella Filipjev, 1927
Metacylicolaimus Schuur.-Stekh., 1946

Paranticoma Micol. & Kreis, 1930
Platycoma Cobb, 1893
Platycomopsis Ditlevsen, 1926
(syn. *Dactylonema* Filipjev, 1927)
Stenolaimus Marion, 1870
Synonchus Cobb, 1893
(syn. *Fiacra* Southern, 1914)
Thoracostoma Marion, 1870
(syn. *Deontostoma* Filipjev, 1916)
Triodontolaimus de Man, 1893
Tubolaimella Cobb, 1933

Genus doubtfully included—
Xennella Cobb, 1920

Subfamily Phanodermatinae Filipjev, 1927

Enoplidae. Amphids not elongate; mandibles absent; stoma rudimentary; oesophagus conoid, posterior part crenate in outline, muscles reduced; 10 cephalic setae; male with one or two tuboid supplements. Type genus: *Phanoderma* Bastian, 1865 (syn. *Cophonchus* Cobb, 1920 and *Heterocephalus* Marion, 1870).

Other genera—

Crenopharynx Filipjev, 1934
Klugea Filipjev, 1927
(? syn. *Gullmarnia* Allgén, 1929)
Micoletzkia Ditlevsen, 1926
Nasinema Filipjev, 1927

Phanodermatina Allgén, 1939
Phanodermella Kreis, 1928
Phanodermopsis Ditlevsen, 1926
(syn. *Galeonema* Filipjev, 1927)

Subfamily OXYSTOMININAE Micol. 1924

Enoplidae. Stoma unarmed; oesophagus conoid with smooth outline; musculature weak; cephalic setae usually 6, post cephalic setae 4; amphids usually elongate, often tuboid internally. Males without preanal supplements. Type genus: *Oxystoma* Bütschli, 1874 with two subgenera *Oxystoma* *s. str.* (syn. *Oxystomatina* Filipjev, 1918; *Acoma* Steiner, 1916; *Schistodera* Cobb, 1920; and *Nemanemella* Filipjev, 1927) and *Nemanema* Cobb, 1920.

Other genera—

Augustinema Cobb, 1933	*Porocoma* Cobb, 1920
Halalaimoides Cobb, 1933	*Thalassoalaimus* de Man, 1893
Halalaimus de Man, 1888	*Trefusia* de Man, 1893

with subgenera—

Halalaimus s. str.	*Tycnodora* Cobb, 1902
(syn. *Nuada* Southern, 1914)	*Pachydora* Wieser, 1953

Doubtful genera—

Asymmetrica Kreis, 1929	*Paroxystomatina* Micol., 1922

Family LAURATONEMATIDAE Gerlach, 1954

Enoploidea. Stoma lightly to moderately sclerotised, unarmed; oesophagus cylindroid, 10 cephalic setae, ovary single, anterior; uterus discharges into cloaca, no externally opening vulva. Males with one testis, no supplements or gubernaculum; spicules straight or nearly so. Cuticle distinctly annulated. Type genus: *Lauratonema* Gerlach, 1954.

Family ONCHOLAIMIDAE Baylis & Daubney, 1926

Enoploidea. Stomatorhabdions heavily sclerotised; stoma somewhat capsuliform, only the posterior part surrounded by oesophageal tissue. Type genus: *Oncholaimus* Dujardin, 1845.

Subfamily ONCHOLAIMINAE Micol. 1922

Oncholaimidae. Oesophagus cylindroid, never crenate or multibulbar; supplement absent or pedunculate. Type genus: *Oncholaimus* Dujardin, 1845.

Other genera—

Adoncholaimus Filipjev, 1918
Anoncholaimus Cobb, 1920
Anoncholaimus Schuur.-Stekh., 1950
Anoplostoma Bütschli, 1874
Cacolaimus Kreis, 1932
Convexolaimus Kreis, 1928
Dioncholaimus Kreis, 1932
Doryonchus Kreis, 1932
Filipjevia Ditlevsen, 1928
Filoncholaimus Filipjev, 1927
Krampia Ditlevsen, 1928
Meroviscosia Kreis, 1932
Metaparoncholaimus de Coninck &
   Schuur.-Stekh. 1933
Metoncholaimoides Wieser, 1953
Metoncholaimus Filipjev, 1918
Mononcholaimus Kreis, 1924
Mononchulus Cobb, 1918

Octonchus nom. nov. pro
Polydontus Schultz, 1932, see below
Oncholaimellus de Man, 1890
Oncholaimoides Chitwood, 1937
Oncholaimium Cobb, 1930
Pelagonema Cobb, 1893
   (syn. *Cavilaimus* Wieser, 1952)
Pelagonemella Kreis, 1932
Phaenoncholaimus Kreis, 1932
Pontonema Leidy, 1856
   (syn. *Paroncholaimus* Filip., 1916)
Prooncholaimus Micol., 1924
Pseudopelagonema Kreis, 1932
Steineriella Ditlevsen, 1928
Trileptium Cobb, 1933 nom. nov.
pro *Trilepta* Cobb, 1920
Vasculanema Kreis, 1928
Viscosia de Man, 1890

Remarks. Wieser (1953) has already remarked that *Anoncholaimus* Schuur.-Stekh., 1950 is a homonym of *Anoncholaimus* Cobb, 1920. He also suggested that it may be synonymous with *Pelagonema* Cobb, 1893. In the meantime I prefer to leave this name to be dealt with by the next reviser who shall either confirm the synonymy or propose a new name. I propose the new name *Octonchus* for *Polydontus* Schultz, 1932 which is preoccupied by *Polydontus* Blainville, 1826 (a mollusc). *Polydontus marinus* Schultz, 1932 will become *Octonchus marinus* **n. comb.**

Subfamily EURYSTOMINAE Filipjev, 1934

Oncholaimidae. Oesophagus conoid, crenate, or multibulbar; stoma similar in both sexes, large, divided by one or more bands, rings or rows of denticles into two chambers. Large tooth broad with a large base. Spicules short, arcuate, gubernaculum usually with a dorsal apophysis; males with two (rarely one or none) cup-like sclerotised supplements. Amphid apertures transverse, oval or reniform. Type genus: *Eurystomina* Filipjev, 1918.

Other genera—

Bolbella Cobb, 1920
Ditlevsenella Filipjev, 1927
Ledovitia Filipjev, 1927

Megeurystomina Luc & de Coninck,
   1959
Pareurystomina Micol. & Kreis, 1930
Thöonchus Cobb, 1920

Subfamily ENCHILIDIINAE Micol., 1924

Oncholaimidae. Oesophagus conoid, sometimes crenate or multibulbar; showing sexual dimorphism in the stomatal region, males without a stoma, females and larvae generally with a stoma divided by a number of trans-

verse rings (sometimes faint and irregular giving the impression of two chambers in *Calyptronema*) into several chambers. Large tooth of female slender, spine-like with a narrow base. Amphid aperture roundish or longitudinal. Spicules elongated, gubernaculum without apophysis. Preanal supplements papilloid. Type genus: *Enchilidium* Nordman, 1840 (nom. nov. pro *Enchelidium* Ehrenb., 1836).

Other genera—

Calyptronema Marion, 1870
   with two subgenera—
Calyptronema s. str.
(syn. *Catalaimus* Cobb, 1920
Rhinoplostoma Allgen, 1929)
Dilaimus Filipjev, 1927
(syn. *Bradystoma* Schuur.-Stekh., 1943)

Conistomella Schuur-Stekh., 1942
Enchilidiella Allgen, 1954
Polygastrophora de Man, 1922
(syn. *Polygastrophorella* Schuur.-Stekh., 1935)
Symplocostoma Bastian, 1865
Symplocostomella Micol., & Kreis, 1930

Superfamily TRIPYLOIDEA Chitwood, 1937

Enoplina. Cuticle of head not duplicate; dorsal and at least two of the subventral oesophageal gland cells discharge anteriorly to the nerve ring (the situation in *Cryptonchus* is not clear). Supplements one or more, often papilloid. Habitat – marine, brackish, or freshwater, and soil. Type genus: *Tripyla* Bastian, 1865.

Family TRIPYLIDAE Oerly, 1880

Tripyloidea. Dorsal and two subventral glands opening in stomatal region. Stomatal walls not heavily sclerotised, stoma either funnel-shaped, or a slender tube with an inconspicuous median dorsal tooth; oesophago-intestinal valve large. Spicules almost straight, gubernaculum present; supplements three or more; caudal glands present. Type genus: *Tripyla* Bastian, 1865. (syn. *Trischistoma* Cobb, 1913).

Other genus: *Tobrilus* Andrássy, 1959 (nom. nov. pro *Trilobus* Bastian, 1865 preocc.).

Family IRONIDAE de Man, 1876

Tripyloidea. Dorsal and subventral glands open near stoma; stoma well developed, heavily sclerotised, cylindrical; with or without setae; amphids pocket-like; one preanal seta present in some males; gubernaculum present. Type genus: *Ironus* Bastian, 1865.

Subfamily IRONINAE Micol., 1922

Ironidae. With three or more teeth at anterior end of stoma; cephalic setae sometimes present; oesophago-intestinal valve small. Type genus: *Ironus* Bastian, 1865 (syn. *Cephalonema* Cobb, 1893).

Other genera—

Conilia Gerlach, 1954
Dolicholaimus de Man, 1888
Ironella Cobb, 1920
Parironus Micol., 1930

Syringolaimus de Man, 1889
Thalassironus de Man, 1889
Trissonchulus Cobb, 1920

Subfamily CRYPTONCHINAE Chitwood, 1937

Ironidae. With one or two small teeth at the base of the stoma; no cephalic setae. Fresh water forms. Type genus: *Cryptonchus* Cobb, 1913 (syn. *Ditlevsenia* Micol., 1925).

Suborder ALAIMINA n. suborder

Enoplida. Stoma vestigial, unarmed; amphids with crescentic apertures (*Amphidelus*), or minute, pore-like (*Alaimus*), commonly placed far back from lip region. Oesophagus dorylaimoid, enlarged only in terminal third. No definite prerectum. Testis single, adanal supplements absent, spicules tripyloid, no gubernaculum. Type genus: *Alaimus* de Man, 1880.

Family ALAIMIDAE Micol., 1922

Alaimina defined as above. Type genus: *Alaimus* de Man, 1880.

Other genus: *Amphidelus* Thorne, 1939

Doubtful genus: *Adorus* Thorne, 1939

Suborder DORYLAIMINA

Enoplida. Setae absent. All oesophageal gland duct orifices posterior to nerve ring; oesophageal glands uninucleate, contained within the wall of the oesophagus; oesophagus cylindroid (Mononchoidea), or basically consisting of a slender anterior part and an expanded posterior part, typically without valves, rarely bi-bulbar (Nygolaimellinae). Oesphagus never degenerate. Axial stylet or mural tooth or teeth present. Males with ventromedian supplements and two spicules. Caudal glands present in Mononchoidea only. Excretory pore generally absent or rudimentary except in Diphtherophoroidea. Mostly free-living in soil or fresh water, rarely marine (some Dorylaiminae) or plant parasitic (some Tylencholaiminae and Diphtherophoroidea).

Superfamily MONONCHOIDEA n. superfam.

Dorylaimina. Oesophagus cylindroid, widening slightly from anterior to posterior end, one dorsal and four subventral, uninucleate oesophageal gland cells. Stoma sclerotised bearing a mural tooth or teeth. Caudal glands typically present. Type genus: *Mononchus* Bastian, 1865.

Family MONONCHIDAE Chitwood, 1937

Mononchoidea. Stoma large, barrel- or cup-shaped, heavily sclerotised, bearing an immovable dorsal tooth with or without subventral teeth, and/or denticles. Amphids small, apertures slit-like. Gubernaculum present, complex lateral accessory pieces commonly present. Caudal glands usually present. Predacious nematodes free-living in soil or freshwater. Type genus: *Mononchus* Bastian, 1865.

Other genera—

Anatonchus (Cobb, 1916) de Coninck, 1939
Brachonchulus Andrássy, 1958
Cobbonchus Andrássy, 1958
Granonchulus Andrássy, 1958
Iotonchus (Cobb, 1916) Altherr, 1950
Judonchulus Andrássy, 1958
Mylonchulus (Cobb, 1916) Pennak, 1953
Miconchus Andrássy, 1958
Prionchulus (Cobb, 1916) Chitw. & Chitw., 1937
Sporonchulus (Cobb, 1916) Pennak, 1953

Family BATHYODONTIDAE n. fam.

Mononchoidea. No setae. Stoma cylindrical, narrow, composed of anterior and posterior sets of three abutting plates (as in *Mononchus*), the anterior ones more heavily sclerotised than the posterior ones. The posterior elements, or the posterior limit of the anterior ones bearing a single tooth. Tooth left subventral in *Miraliamus* and *Bathyodontus*, and reputedly dorsal in *Oionchus*. Amphids cup-shaped, small. Ovaries single or paired, reflexed; tail short, rounded; caudal glands and terminal duct present. Only females known. Free-living in soil. Type genus: *Bathyodontus* Fielding, 1950.

Other genera—

Mirolaimus Andrássy, 1956   *Oionchus* Cobb, 1913

Remarks. This new family is erected for the reception of genera previously assigned to the Nygolaiminae. They differ from other genera in that subfamily in the form of the oesophagus and the presence of caudal glands and duct. Observations on living *Mirolaimus* confirm that the caudal gland acts as an adhesive organ. In *en face* view the similarity of *Mirolaimus* and the mononchids is remarkable. *Oionchus* alone possesses the glands at the oesphago-intestinal junction which are also found in the Nygolaims. There are indications that the Bathyodontidae may represent a transitional group between the Mononchoidea and the Dorylaimoidea, and that their kinship lies with the Nygolaimidae rather than with the Dorylaimidae. Knowledge of the males is desirable before this line of argument is pursued. *Enoplochilus* Kreis, 1932, once thought to be close to *Oionchus* is not placed in this classification. It may belong near the Eurystominae on the evidence of the reniform amphid, but it has nothing to do with the Dorylaimina.

Superfamily DORYLAIMOIDEA Thorne, 1934

Dorylaimina. Oesophagus basically consisting of a slender anterior part and an expanded posterior part; cephalic papillae in two rings, 6 in the inner and 10 in the outer; amphid apertures posterior to the cephalic papillae. Amphids stirrup-shaped or pouch-like with slit-like or ellipsoidal apertures. Stoma armed with an axial spear with a dorsal aperture, or a mural tooth. Lateral pores and prerectum present. Supplements consisting of a ventromedian series and an adanal pair. Testes two. No caudal glands.

Family DORYLAIMIDAE de Man, 1876

Dorylaimoidea. Polymyarian. Amphids with obscure slit-like apertures. Stoma with an axial spear; oesophagus enlarged in posterior third or more, enlarged part not surrounded by a spiral sheath of connective tissue (a nonspiral sheath in *Drepanodorus striatus* (Krall, 1958)). Cuticle generally with minute transverse striae, sometimes with wing-like longitudinal striae over the whole body (certain species of *Dorylaimus* and *Actinolaimus*). Spicules with lateral guiding pieces; gubernaculum absent. Type genus: *Dorylaimus* Dujardin, 1845.

Subfamily DORYLAMINAE Filipjev, 1918

Dorylaimidae. Spear axial, spear or extensions not greatly elongated, without basal knobs, flanges, or tripartite extensions. Ovaries one or two. (Only species with sheath of connective tissue about enlarged part of oesophagus *Drepanodorus striatus* (Krall, 1958) which is characterised by also having the spear aperture longer than half the spear length.) Type genus: *Dorylaimus* Dujardin, 1845.

Other genera—

Amphidorylaimus Andrássy, 1959
Aporcelaimus Thorne & Swanger, 1936
Chrysonema Thorne, 1929
Discolaimium Thorne, 1939
Discolaimus Cobb, 1919
Drepanodorus Altherr, 1954
(n. syn. *Paraxonchium* Krall, 1958)
Eudorylaimus Andrássy, 1959
Labronema Thorne, 1939

Lordellonema Andrássy, 1960
Mesodorylaimus Andrássy, 1959
Meylonema Andrássy, 1950
Prodorylaimus Andrássy, 1959
Pungentus Thorne & Swanger, 1936
Thornia Meyl, 1954
Thorneella Andrássy, 1960
Thornema Andrássy, 1959
Wittoldinema Brzeski, 1960

Remarks. The inclusion of *Paraxonchium striatum* Krall = *Drepanodorus striatus* (Krall, 1958) n. syn. in the Dorylaiminae is problematical. In conjunction with Dr J. B. Goodey it has been decided that because this species is so similar to other species referred to *Drepanodorus,* apart from the presence of the sheath about the expanded part of the oesophagus that it is best included here. Authors of the other species of *Drepanodorus* (Altherr, 1954 and Andrássy, 1956) did not record any such sheath.

Subfamily TYLENCHOLAIMINAE Filipjev, 1934

(including Longidorinae Thorne, 1935)

Dorylaimidae. Spear short with knobbed or flanged spear extensions, or greatly elongated with long, rod-like, or flanged extensions. Ovaries one or two. Tail similar in both sexes where known, except *Miranema*. Type genus: *Tylencholaimus* de Man, 1876.

Other genera—

Discomyctus Thorne, 1939   *Miranema* Thorne, 1939
Enchodelus Thorne, 1939   *Utahnema* Thorne, 1939
Longidorella Thorne, 1939   *Xiphinema* Cobb, 1913
Longidorus Micol., 1922   *Xiphinemella* Loos, 1950
                                     (*syn. Taprobanus* Loos, 1949)

Remarks. *Tylencholaimus bryophilus* Imamura, 1931 was referred by Thorne, 1939 to *Doryllium* Cobb, 1920. It does not conform with Thorne's diagnosis of the genus because of its prodelphic ovary, and the large number of ventro-median supplements. The fine spear with its minute knobs contrasts strikingly with the other species referred to the genus. The shape of the oesophagus as figured by Imamura (1931) appears leptonchid, but may be interpreted as a further development of the type of oesophagus known in *Tylencholaimus nikkoensis* Kaburaki & Imamura, 1933 which was retained within the Tylencholaiminae by Thorne. Whilst the systematic position of *T. bryophilus* is difficult to determine I prefer on the basis of the spear, ovaries, and supplements to place this species once more in the genus *Tylencholaimus* de Man, 1876.

It may be noted that the original figures of *T. nikkoensis* and *T. kirifuri* Kaburaki & Imamura, 1933 differ in a number of important diagnostic features from the figures in Thorne, 1939.

Subfamily ACTINOLAIMINAE Thorne, 1939

Dorylaimidae. Walls of stoma partially or completely sclerotised, frequently bearing onchia or denticles. Supplements sometimes grouped into fascicles. Type genus: *Actinolaimus* Cobb, 1913, (= *Brasilaimus* Lordello & Zamith, 1957 n. syn.).

Other genera—

Actinolaimoides Meyl, 1957   *Mylodiscus* Thorne, 1939
Antholaimus Cobb, 1913   *Paractinolaimus* Meyl, 1957
Carcharolaimus Thorne, 1939   *Trachypleurosum* Andrássy, 1959 nom.
Metactinolaimus Meyl, 1957   nov. pro. *Trachypleura* Thorne,
                                        1939, preocc.

Remarks. *Brasilaimus* Lordello & Zamith, 1957 is indistinguishable from *Actinolaimus* Cobb, 1913 on the basis of the information supplied in the generic diagnosis, or in the description and figures of the type species

B. subaquilus L. & Z., 1957. *Brasilaimus* is therefore synonymised with *Actinolaimus* Cobb, 1913. *B. subaquilus* becomes *Actinolaimus subaquilus* (Lordello & Zamith, 1957) **n. comb.**

Family Opailaimidae Kirjanova, 1951

Dorylaimoidea. Amphids and sensillae very large, expanded; posterior half of the oesophagus surrounded by a "cuticular" sheath. Spear long fine, delicate, unlike that of the Dorylaimidae. Type genus: *Opailamus* Kirjanova, 1951.

Other genus: *Ottolaimus* Kirjanova, 1951.

Remarks: The above diagnosis is a free paraphrasing of Kirjanova (1951). The exact status of this family is rather doubtful. Neither of the two species referred to it is adequately described or figured for species which reputedly possess so many outstanding characters. The arrangement of the spear, guiding ring, and amphid apertures of *Opailaimus* appears most unusual. *Ottolaimus* could well be based on a moulting specimen of *Discolaimus*. Both genera urgently need careful study.

Family Belondiridae Thorne, 1939

Dorylaimoidea. Polymyarian. Spear axial, small, often slightly arcuate in lateral view, dorsal aperture never distinctly longer than half spear length; spear extensions in two obscurely separated sections which bear broad flanges. Basal enlargement of oesophagus very variable in size, always surrounded by a sheath of spiral connective tissue. Ovaries paired or single; anterior ovary usually vestigial or absent. Supplements an adanal pair and a ventro-median series. Lateral guiding pieces present except in *Dorylaimellus*. Tails of both sexes similar where known. Type genus: *Belondira* Thorne, 1939.

Other genera—

Axonchium Cobb, 1920   *Oxydirus* Thorne, 1939
Dorylaimellus Cobb, 1913   *Swangeria* Thorne, 1939
(syn. *Pungentoides* Altherr, 1950)

Remarks. See under Nygolaimidae.

Family Nygolaimidae Thorne, 1939 n. grad.

Dorylaimoidea. Polymyarian. Spear a left subventral mural tooth set in an eversible stoma. Oesophagus usually expanded in basal half or more, rarely bi-bulbar (Nygolaimellinae); with or without a spiral sheath of connective tissue about expanded part. Three glandular bodies present at oesophago-intestinal junction. Ovaries paired or single, reflexed. Lateral guiding pieces and gubernaculum present in all known males. Type genus: *Nygolaimus* Cobb, 1913.

Remarks. I have somewhat changed the diagnoses of the families Belondi-
ridae and Nygolaimidae, and the emphasis that Thorne placed on certain
characters. The Nygolaimidae are characterised by the presence of a ventro-
lateral mural tooth, with or without a spiral oesophageal sheath, whereas
the Belondiridae are characterised by the presence of an axial spear and
the spiral sheath of connective tissue about the base of the oesophagus. It
is largely on these grounds that I have removed the genera *Nygellus* and
Nygolaimellus from the Belondiridae and assigned them to the Nygolai-
midae. It should also be noted that both genera possess the three glandular
bodies at the oesophago-intestinal junction, a feature found in the Nygo-
laimidae but not in the Belondiridae. Thorne may have been influenced in
placing *Nygellus* with the Belondirids by the single posterior ovary in
N. clavatus Thorne, but *N. symmetricus* Williams, 1958 shows that this
character is not constant within the genus.

See also remarks on the Bathyodontidae.

Subfamily NYGOLAIMINAE Thorne, 1939

Nygolaimidae. Oesophagus unibulbar, with or without a spiral sheath about
oesophagus base. Type genus: *Nygolaimus* Cobb, 1913.

Other genera—
Heterodorus Altherr, 1952   *Sectonema* Thorne, 1930
Nygellus Thorne, 1939

Subfamily NYGOLAIMELLINAE n. subfam.

Nygolaimidae. Oesophagus bibulbar, with a sheath about posterior part of
oesophagus. Type genus: *Nygolaimellus* Loos, 1949.

Family LEPTONCHIDAE Thorne, 1935

Dorylaimoidea. Meromyarian. Spear axial, basal extensions well developed,
or bearing basal knobs or flanges. Amphids large, stirrup shaped, aperture
rather narrow in some *Tylencholaimellus* spp. Oesophagus slender with
a short expanded basal region (rarely as much as one third of oesophageal
length in some *Dorylaimoides*), which may be set off by a marked con-
striction. Lateral series of pores in two lines. Prerectum present. Males
where known with lateral guiding pieces; no gubernaculum. Type genus:
Leptonchus Cobb, 1920.

Other genera—
Dorylaimoides Thorne & Sw., 1936   *Proleptonchus* Lordello, 1953
Doryllium Cobb, 1920   *Tylencholaimellus* Cobb, 1915

Remarks. I am not convinced that the Leptonchidae form a coherent
natural group, nor that these meromyarian types naturally belong within

the Dorylaimoidea which are essentially polymyarian. Opinion is divided on the value of this feature as a taxonomic criterion. When more forms are known it may be possible to define and subdivide the group with more certainty. In the meantime I have removed the Campydorinae from this family.

Family CAMPYDORIDAE Thorne, 1935 n. grad. Emend.

Dorylaimoidea. Meromyarian. Oesophagus slender in anterior four-fifths, small basal bulb, often set off by a marked constriction, with a triquetrous valvular chamber. Amphids stirrup-shaped; spear axial, with or without flanged basal extensions or a dorsal mural tooth. Ovary single, either pro- or opistho-delphic. Prerectum and excretory pore present or absent. Type genus: *Campydora* Cobb, 1920.

Other genera—

Aulolaimoides Micol., 1914   *Tyleptus* Thorne, 1939

Remarks. This family as it now stands is a "taxonomic expediency". Its chief purpose is to gather together the three genera that have a valve-like chamber in a small posterior oesophageal bulb, and which do not fit into any other family. As more species and genera with this character become known it will become necessary to subdivide it, a practice which has little to commend it at the present time.

Superfamily DIPHTHEROPHOROIDEA n. superfam.

Dorylaimina. Meromyarian. Spear short, stout, complex, or long, arcuate, attenuated, only tip moulted. Amphid apertures ellipsoidal. Anatomical details generally obscured by abundant granules. Oesophagus with a pyriform to elongate conoid basal bulb. Prerectum absent; testis one; ventro-median supplements present, adanal pair absent; gubernaculum present. Type genus: *Diphtherophora* de Man, 1880.

Family DIPHTHEROPHORIDAE Thorne, 1935 Emend

Diphtherophoroidea. Spear with basal knobs, stout, posteriorly of complex archlike structure in anterior part of dorsal sector, ventral sector apparently not fused to dorsal sector posteriorly. Guiding apparatus of spear a complicated structure of plates and rods of which an inverted V-shaped piece is often the most conspicuous. Supplements often reduced or vestigial. Type genus: *Diphtherophora* de Man, 1880.

Other genera—

Brachynemella Cobb, 1933   Triplonchium Cobb, 1920
(syn. Brachynema Cobb, 1893 pre-   Tylolaimophorus de Man, 1880
    occ.)

Family TRICHODORIDAE Thorne, 1935 n. grad.

Diphtherophoroidea.   Spear strictly a dorsal tooth, long, attenuated, arcuate, without basal knobs (often described as tripartite in the middle region but this is a misinterpretation – see below). Ovaries paired or single, reflexed when paired; males with or without caudal alae; spicules straight or nearly so. Tail length not more than one anal-body-width. Type genus: *Trichodorus* Cobb, 1913.

Remarks.   Allen (1957) has monographed this group and has figured the cross section of the "tripartite" part of the spear as U-shaped. He has informed me (in litt.) that the tripartite appearance is due to the greater passage of light through the centre of the spear when it is viewed laterally.

Suborder TRICHOSYRINGINA Ward, 1917

Enoplida.   Setae absent; oesophageal glands forming a stichosome outside the contour of the oesophagus. Amphids pocket-like (some Mermithoidea) to pore-like. Parasitic at some stage of the life history in terrestrial or freshwater arthropods (Mermithoidea) or in vertebrates (Trichuroidea). Type genus: *Trichuris* Roederer, 1761.

Superfamily MERMITHOIDEA Wülker, 1924

Trichosyringina.   Amphids modified pocket-like to pore-like; intestine extending anterior to base of oesophagus, usually without a lumen. Male with one or two spicules, usually two testes. Female reproductive system highly developed, usually with two ovaries and tubular vagina. Eggs modified, but not operculate. Bursa present in some *Eurymermis*. Type genus: *Mermis* Dujardin, 1842.

Family MERMITHIDAE Braun, 1883

Mermithoidea.   Oesophagus not dorylaimoid in larval stages; larvae with an axial spear. Adults long slender worms with a smooth cuticle. Adult intestine replaced by trophosome or pseudo-intestine. Anus non functional. Larvae parasitic in freshwater and terrestrial arthropods. Adults free-living. Type genus: *Mermis* Dujardin, 1842.

The following genera have been erected—

Agamermis Cobb, Steiner & Christie, 1923
Agamomermis Stiles, 1903
Allomermis Steiner, 1924
Amphidomermis Filipjev, 1934
Amphimermis Kaburaki & Imamura, 1932
Bathymermis Daday, 1911
? *Bolbinium* Cobb, 1920
Complexomermis Filipjev, 1934
Eomermis Steiner, 1925
Eumermis Daday, 1911
Eurymermis Müller, 1931
Filipjevimermis Polozhentsev & Artyukhovsky, 1958
Gastromermis Micol., 1925
Gordiomermis Heinze, 1934
Hexamermis Steiner, 1934
Heydonius Taylor, 1935
Hydromermis Corti, 1902
Isomermis Coman, 1953
Limnomermis Daday, 1911
Megalomermis Müller, 1931
Mesomermis Daday, 1911
Nanomermis Cobb, 1924
Neomermis Linstow, 1904
(syn. *Octomermis* Steiner, 1929)
Paramermis Linstow, 1898
Phreatomermis Coman, 1953
Psammomermis Polzhentsev, 1941
Pseudomermis de Man, 1903
Skrjabinomermis Polozhentsev, 1952
Tetramermis Steiner, 1925
Tunicamermis Schuur-Stekh., Mawson, & Couturier, 1955

Remarks.   The above list of generic names was supplied by Dr H. E. Welch, who has in preparation a critical revision of the Mermithidae which may result in the synonymisation or rejection of some of the generic names listed above.

Family TETRADONEMATIDAE Cobb, 1919

Mermithoidea.   Oesophagus dorylaimoid in larvae. All stages of life history may be found in insects; no rectum or anus in adults; males with one spicule, with or without supplements. Type genus: *Tetradonema* Cobb, 1919.

Other genera—

Aproctonema Keilin, 1917   *Mermithonema* Goodey, 1941

Superfamily TRICHUROIDEA Railliet, 1915

syn. Trichocephalata Skrjabin & Schultz, 1928

Trichosyringina. Amphids pore like; a spear present in larvae, rarely present in adults; intestine not extending anterior to base of oesophagus, lumen well developed, rarely terminating blindly (Cystoopsidae). Males with one testis and one or no spicules. Female with tubular vagina, one ovary; eggs typically operculate. Parasites of vertebrates in adult stages. Life history direct or indirect. Type genus: *Trichuris* Roederer, 1761.

Remarks.   I have retained the more familiar name for this superfamily for the present. Although Leiper (1926) pointed out that *Trichuris* Roederer, 1761 is not available under the Rules, and that the correct name should be

Trichocephalus Goeze, 1882, most workers have been loath to adopt it. Under the Copenhagen Decisions on zoological nomenclature there is no longer any need to change family names which have been based on junior synonyms. The situation is not clear in this case as Trichuris is not strictly a synonym as it has never been legally available.

Family TRICHURIDAE Railliet, 1915

Trichuroidea. Oviparous, stichosome one row of stichocytes. Males with one spicule. Type genus: *Trichuris* Roederer, 1761.

Subfamily TRICHURINAE Ransom, 1911

Trichuridae. Posterior part of body distinctly wider than anterior part; oesophageal region much longer than rest of body. Type genus: *Trichuris* Roederer, 1761 (syn. *Trichocephalus* Goeze, 1882).

Subfamily CAPILLARIINAE Railliet, 1915

Trichuridae. Posterior part of body not distinctly wider than anterior part; oesophageal region not distinctly longer than rest of body. Type genus: *Capillaria* Zeder, 1800 (syn. *Aonchotheca* Lopez-Neyra, 1947, *Capillo-strongyloides* Freitas & Lent, 1935).

Other genera—

Eucoleus Dujardin, 1845   *Skrjabinocapillaria* Skarbilovitch, 1946
Hepaticola Hall, 1916   *Thominx* Dujardin, 1845
                                           (syn.   *Echinocoleus*   Lopez-Neyra,
                                              1947)

Family TRICHOSOMOIDIDAE York & Maplestone, 1926

Trichuroidea. Oesophageal region roughly equal to, or shorter than remainder of body; spear present or absent in adults. Males without spicules or copulatory sheath; either much smaller than female and parasitic within uterus or vagina, or non-parasitic and equal in size to female. Females oviparous or ovoviviparous. Type genus: *Trichosomoides* Railliet, 1895.

Subfamily TRICHOSOMOIDINAE Hall, 1916

Trichosomoididae. Oesophagus about equal to rest of body. Spear absent in adults. Males minute, parasitic within uterus of female. Type genus: *Trichosomoides* Railliet, 1895.

Other genus—
Trichuroides Ricci, 1949.

Subfamily ANATRICHOSOMATINAE Smith & Chitw., 1954

Trichosomoididae. Oesophagus one-third to one-sixth of body-length. Males not parasitic in female, size equal to or larger than females. Adults with spears. Parasites of monkeys. Type genus: *Anatrichosoma* Smith & Chitwood, 1957.

Family TRICHINELLIDAE Ward, 1907

Trichuroidea. Ovoviviparous; stichosome one row of stichocytes; males not degenerate; without spicules. Vulva in middle of oesophageal region. Oesophagus more than half body length in female, less than half body length in male. Type genus: *Trichinella* Railliet, 1895 (syn. *Trichina* Owen, 1835 nec Meigen, 1830).

Family CYSTOOPSIDAE Skrjabin, 1923

Trichuroidea. Oviparous. Stichosome two rows of stichocytes. Males with one spicule. Vulva in oesophageal region. (Only one species which is parasitic in the skin of the Volga sturgeon.) Type genus: *Cystoopsis* Wagner, 1867.

Suborder DIOCTOPHYMATINA

Enoplida. Setae absent, but long papillae may be present in larval stages. Oesophageal glands highly polynucleate, branching dichotomously within the contour of the oesophagus, ducts opening anterior to nerve ring. No lips; no spear in adults; oesophagus cylindroid, reproductive system single in both sexes. Males with one spicule in caudal sucker. Eggs operculate. Adults parasites of vertebrates.

Family DIOCTOPHYMATIDAE Railliet, 1915

Dioctophymatina. Muscular cephalic sucker absent. Type genus: *Dioctophyme* Collet-Meygret, 1802.

Subfamily DIOCTOPHYMATINAE Cast. & Chambers, 1910

Dioctophymatidae. Very large nematodes, females up to one metre long, males one-third of this. Vulva in anterior part of body. Adults parasitic in mammals. Type genus: *Dioctophyme* Collet-Meygret, 1802.

Other genus: *Mirandonema* Kreis, 1945.

Subfamily EUSTRONGYLINAE Chitw. & Chitw., 1937

Dioctophymatidae. Moderate to large size, cuticle coarsely striated towards extremities, with or without spines. Vulva near anus. Adults parasitic in birds. Type genus: *Eustrongylides* Jägerskiöld, 1909.

Other genus: *Hystrichis* Dujardin, 1845.

Family SOBOLIPHYMATIDAE Petrov, 1930

Dioctophymatina.   With large muscular cephalic sucker. Females oviparous, with vulva near anus. Adults parasitic in carnivores and fish. Type genus: *Soboliphyme* Petrov, 1930.

KEY TO FAMILIES AND SUBFAMILIES OF THE ENOPLIDA

Taxonomic keys are attempts at finding short cuts, and like other short cuts they have their dangers. All identifications, at even the higher levels, must be checked against full descriptions and figures. In this key items in italics are spot characters, if they can be determined with certainty the remainder of the couplet may be ignored. References in brackets refer to published keys to genera.

1. Oesophageal glands within contour of oesophagus		9
Oesophageal glands present as a stichosome outside oesophageal contour	TRICHOSYRINGINA	2
2. Intestine extending anterior to base of oesophagus, usually without a lumen	MERMITHOIDEA	3
Intestine not extending anterior to base of oeso-phagus, lumen well developed	TRICHUROIDEA	4
3. Oesophagus dorylaimoid in larval stages; adults and larvae found in insects. Males with one spicule	TETRADONEMATIDAE	
Oesophagus not dorylaimoid in larval stages; only larvae parasitic in terrestrial and freshwater arthro-pods; males with one or two spicules	MERMITHIDAE	
4. Stichosome one row of stichocytes		5
Stichosome two rows of stichocytes	CYSTOOPSIDAE	
5. Males degenerate living within the uterus or vagina of the female, or *lacking spicules* and couplatory sheath		6
Males not degenerate, *with one spicule*		8
6. *Males degenerate, living within female uterus* or vagina	TRICHOSOMOIDINAE	
Males not degenerate, not living within female		7
7. *Adults with spear,* vulva near oesophago-intestinal junction	ANATRICHOSOMATINAE	
Adults without spear, vulva in middle of oesophageal region	TRICHINELLIDAE	
8. Oesophageal region extremely slender, longer than remainder of body	TRICHURINAE	
Oesophageal region shorter than rest of body and not remarkably slender	CAPILLARIINAE	
9. Very large nematodes, adults parasitic in vertebrates; *males with caudal sucker* and one spicule; eggs oper-culate	DIOCTOPHYMATINA	10
Not very large; not parasitic in vertebrates, males without caudal sucker; eggs not operculate		12

Sig. 10

10. With muscular cephalic sucker — SOBOLIPHYMATIDAE
    Without muscular cephalic sucker — DIOCTOPHYMATIDAE　11

11. Vulva in anterior part of body — DIOCTOPHYMATINAE
    Vulva in posterior part of body — EUSTRONGYLIDINAE

12. Stoma vestigial, without spear or teeth, cephalic setae absent, oesophagus dorylaimoid, males without adanal supplements or gubernaculum (Thorne, 1939) — ALAIMIDAE
    Not as above — 13

13. *All oesophageal gland ducts posterior to nerve ring*, no cephalic setae, oesophagus cylindroid, dorylaimoid or bi-bulbar; axial spear or mural tooth or teeth present, rarely marine — some Dorylaiminae. Caudal glands present in Mononchoidea only — DORYLAIMINA　14

    Some or all oesophageal gland ducts anterior to nerve ring; usually with cephalic setae, oesophagus cylindroid, crenate, conoid or multibulbar. Caudal glands usually present — ENOPLINA　28

14. Oesophagus cylindroid — MONONCHOIDEA　15
    Oesophagus dorylaimoid, conoid, or bi-bulbar — 16

15. Stoma heavily sclerotised, cup- or barrel-shaped; armed with one or three teeth, tail conoid, arcuate, or filiform　(Andrássy, 1958) — MONONCHIDAE
    Stoma moderately sclerotised; elongate, narrow; tail short, rounded, not conoid or filiform — BATHYODONTIDAE

16. Spear short, stout with dorsal and ventral elements apparently separate posteriorly, or long, slender arcuate without dorsal aperture; prerectum, lateral pores, and *adanal supplements absent*; *one testis*; excretory pore usually visible; anus often almost terminal — DIPHTHEROPHOROIDEA　17
    Spear a hollow tube with an oblique dorsal aperture (aperture may be absent when spear is mural), often with variously developed spear extensions. *Testes two*; *adanal supplements*, lateral pores, and usually prerectum present. Excretory pore absent or rudimentary — DORYLAIMOIDEA　18

17. Spear short, complex, with basal knobs; spear guiding apparatus of sclerotised plates and rods (Thorne, 1939) — DIPHTHEROPHORIDAE
    Spear long, arcuate, tapering towards ends, without basal knobs; obscure non-sclerotised guiding ring present (Allen, 1957) — TRICHODORIDAE

18. Oesophageal bulb small, with a triquetrous valvular chamber — CAMPYDORIDAE
    Oesophageal bulb without a triquetrous valvular chamber — 19

19. Spear mural — NYGOLAIMIDAE　20
    Spear axial — 21

20. Oesophagus bi-bulbar — NYGOLAIMELLINAE
    Oesophagus mono-bulbar (dorylaimoid) — NYGOLAIMINAE

21. Posterior part of oesophagus with a spiral sheath of connective tissue; anterior ovary usually vestigial or absent (if spear aperture is more than half spear length c.f. *Drepanodorus* Dorylaiminae) — BELONDIRIDAE
    Oesophagus without a spiral sheath — 22

22. Spear extensions bearing knobs 23
    Spear extensions not bearing knobs, but, may bear
    flanges 24

23. Males with adanal and one ventromedian supplement,
    spear knobs moderate to large, spear with dorsal
    thickening. Females with posterior ovary only,
    anterior uterus greatly reduced or absent .... part of   LEPTONCHIDAE
    Males with adanal and three or more ventromedian
    supplements; spear knobs small, spear not thickened
    dorsally. Females with only anterior or paired ovaries
    (single and posterior only in *Tylencholaimus zee-
    landicus* de Man, 1876) .... part of   TYLENCHOLAIMINAE

24. Oesophagus enlarged in distal third or more 25
    Oesophagus enlarged in less than distal third; pos-
    terior bulb often pyriform or set off by marked
    constriction .... remainder of   LEPTONCHIDAE

25. Spear short and stout with oblique aperture, or if
    slender spear length greater than three times width
    of lip region. Spear often with flanges. Posterior half
    of oesophagus without cuticular sheath   DORYLAIMIDAE   26
    Spear slender tapering uniformly to tip, length less
    than three times width of lip region; spear without
    knobs or flanges. With amphids very large, or with
    two anteriorly projecting "ears" on head. Posterior
    half of oesophagus surrounded by a "cuticular"
    sheath   OPAILAIMIDAE

26. Stomatal region heavily sclerotised, often dentate   ACTINOLAIMINAE
    Stomatal region not heavily sclerotised; never dentate   27

27. Spear and spear extension simple, short or of moder-
    ate length, never bearing flanges, etc (Andrássy,
    1959)   DORYLAIMINAE
    Spear and or extensions greatly attenuated, or spear
    of moderate length with flanged extensions   TYLENCHOLAIMINAE

28. All oesophageal gland ducts opening in stomatal
    region anterior to nerve ring. Cuticle of head dupli-
    cate; supplements 0, 1, or 2   ENOPLOIDEA   28
    Dorsal and two subventral oesophageal glands open-
    ing anterior to nerve ring. Cuticle of head not dupli-
    cate; supplements one or more   TRIPYLOIDEA   37

29. Vagina opening into cloaca, no external vulva   LAURATONEMATIDAE
    Vulva normal, external; vagina not discharging into
    cloaca   30

30. Stomatorhabdions poorly sclerotised; without distinct
    stomatal capsule, stoma surrounded by oesophageal
    tissue, or stoma absent   31
    Stomatorhabdions heavily sclerotised, stoma some-
    what capsuliform; only posterior part surrounded
    by oesophageal tissue   ONCHOLAIMIDAE   35

31. Stoma absent, supplement papilloid .... males of   ENCHILIDIINAE
    Stoma present supplement(s) tuboid or absent   ENOPLIDAE   32

32. Stoma with three bifurcate mandibles   ENOPLINAE
    Stoma without three bifurcate mandibles   33

33. Posterior part of oesophagus crenate in outline, vesi-
    culate, musculature reduced   (Wieser, 1953a)   PHANODERMATINAE
    Posterior part of oesophagus not crenate in outline,
    never vesiculate   34

34. Supplements absent; oesophagus conoid, oesophageal musculature weak; $6 + 4$ setae in external circle (Wieser, 1953a)   OXYSTOMATINAE

     Supplements one or two, tuboid; oesophagus usually cylindroid, rarely conoid; posterior oesophageal musculature well developed. Ten setae in external circle (Wieser, 1953b)   LEPTOSOMATINAE

35. Oesophagus cylindroid, never crenate; supplements absent or pedunculate   (Wieser, 1953a)   ONCHOLAIMINAE

     Oesophagus conoid, crenate or multibulbar; supplements papilloid, cup-like, or rarely absent   36

36. With sexual dimorphism of stoma; males without a stoma. Female stoma generally narrow, divided by a number of transverse rings into several chambers (sometimes rings faint giving the impression of two chambers in *Calyptronema*). Teeth slender, needle-like, base narrow. Spicules elongate, gubernaculum without apophysis; supplements papilloid   (Wieser, 1953a)   ENCHILIDIINAE

     Without sexual dimorphism of stoma; stoma large, divided by one or more bands, rings, or rows of denticles into two chambers. Tooth broad with large base. Spicules short, arcuate, gubernaculum usually with a dorsal apophysis; supplements sclerotised, cup-like when present   (Wieser, 1953a)   EURYSTOMINAE

37. Stoma walls not heavily sclerotised; stoma either funnel-shaped or a very slender tube with an inconspicuous dorsal tooth. Tooth or teeth in median part of stoma. Spicules almost straight   TRIPYLIDAE

     Stoma walls strongly sclerotised, stoma elongate, teeth at anterior or posterior end of stoma. Spicules usually arcuate   IRONIDAE   38

38. Teeth at anterior end of stoma, commonly with cephalic setae   IRONINAE

     Teeth at posterior end of stoma, never with cephalic setae   CRYPTONCHINAE

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AN ELECTRIC FISHING MACHINE WITH PULSATORY DIRECT CURRENT

By A. M. R. Burnet, Fisheries Research Laboratory, Marine Department.

Summary

The electrical constants for fishing with an earth-return system are given. A boat-mounted unit for larger streams is described. It can be used by three operators. Automatic control circuits which give the maximum safety of operation are described.

INTRODUCTION

This paper gives details of circuits and operation of an electro-fishing machine based on the principle of dual frequency pulsatory direct current described by Burnet (1959).

In New Zealand, waters with resistances of from 4,000 ohms per cubic inch to 20,000 ohms per cubic inch are commonly encountered, and provision must be made for a range of output voltages. For smaller streams up to 15 ft wide and not over 2 ft deep, the single positive electrode and earth return system is convenient and satisfactory. In larger streams a multi-electrode system attached to a boat is used. The latter arrangement is also necessary when the ground resistance is high.

The aim has been to obtain a high catch efficiency, as the equipment is used mainly for population estimates and growth studies based on the recapture of tagged fish.

The use of a machine with a low catch efficiency for population estimation by the mark and recapture technique is subject to serious criticism, in view of the present knowledge of the territorial behaviour of trout (Gerking 1959). Such equipment would vary in efficiency from place to place, and, in the absence of a random mixing of the marked fish, the population estimates could be inaccurate.

In all electric fishing operations careful observance of safety precautions is essential; the operators must be familiar with the equipment and the techniques of catching fish. The more they work as a team, anticipating each others movements, the greater the degree of safety. The electrode operator should wear rubber gloves, and the rubber thigh boots or body waders of all operators must be in good condition.

THE EARTH RETURN SYSTEM

The positive electrode is formed from a five-ft length of $\frac{5}{8}$ in. aluminium alloy tubing, and is illustrated in Fig. 1. The earth return comprises 8 sq. ft. of wire gauze which is placed on the stream bed.

Fig. 1—Details of the positive electrode with its switch and meter as used for the earth return system. A wire gauze dip-net is used because fish inside the net are protected from the electric current. Also, it is easier to pick up and tip out fish with a rigid net.

The best results are obtained by working downstream to a stop net. However, as good visibility is essential for successful fishing it is better to work upstream when conditions are such that mud is stirred up by the operators' feet. The positive electrode has been used up to 600 yards from the earth mat with no apparent loss in effectiveness.

Suitable output voltages for waters of various resistances are given in Table 1. These are based on data given by Haskell (1954), Lethlean (1953) and on field experience.

Control Circuit

The cable running to the positive electrode is broken by a waterproof spring-release switch (Sw1) on the electrode handle, as shown in Fig. 2. When this switch is closed it completes the circuit allowing current to
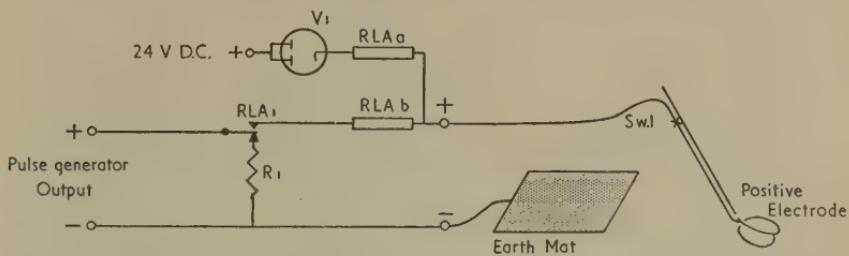
FIG. 2—Diagram of control circuit for an earth return system. Components: R1, load resistor, adjusted to match the load presented by the electrode system; RLA relay, Post Office type 3000 with 5 amp contacts, make and break; SW 1, spring release waterproof switch (micro-switch in metal case operated through a rubber sealed plunger); V 1, electron tube, type 6X4.

flow from a 24 volt source through the relay (RLAa) which operates, and connects the electrode cable to the output of the pulse generator. The pulse current passing through the second coil of the relay (RLAb) assists in keeping the relay closed. When the electrode switch is released, the relay releases, and disconnects the pulse generator output.

An important feature of this arrangement is that when the electrode is lifted out of the water the relay releases, and disconnects the pulse generator output. It is therefore impossible to have a 'live' electrode out of the water. This is a valuable safety feature in that it prevents the most dangerous situation in electric fishing, i.e., a live electrode out of the water. An operator coming in contact with the live electrode in the water would be affected by only a proportion of the total output voltage. When, however, the live electrode is out of the water, the whole of the output voltage appears between the electrode and any point in the water.

The electron tube in the circuit is a one-way device to prevent the pulse generator output from operating the low voltage coil of the relay (RLAa).

A practical advantage of this control system is that only a single wire cable is required for the positive electrode.

The relay is designed so that coil 'a' will operate with a resistance of 1,000 ohms or less in the output. Allowing for internal resistances the relay should operate on a minimum current of 20 milliamperes. Coil 'b' holds the relay closed on the minimum pulse current likely to flow, and must be able to carry the peak pulse current.

The Boat-mounted Electrode System

The two negative electrodes are 6 ft lengths of $\frac{1}{2}$ in. wide metal braid attached to the ends of 8 ft bamboo poles, as shown in Fig. 3. The poles

Fig. 3—The boat-mounted electrode system showing the positions of the negative and positive electrodes.

are attached to the boat by coil springs which are tensioned to keep the poles at right angles to the boat (Fig. 4). They are held in position by a bridle, but will fold back to follow the contour of the stream bank or to pass obstructions. The two positive electrodes are 30 in. lengths of $\frac{5}{8}$ in. diameter aluminium alloy tubing attached to the end of 3 ft arms, which are also held in position by coil springs. Setting the positive electrodes out from the stern of the boat ensures that fish are not stunned under the boat where they could not be seen.

The operator, standing at the stern of the boat has a wire-gauze dip-net which can be used as an additional positive electrode.

The dip-net handle is provided with two spring release switches. Closing Sw2 operates the relay RLB (Fig. 5) to connect the pulse generator to the fixed positive electrode system. The operating lever for this switch is made so that it is conveniently held closed while using the dip-net.
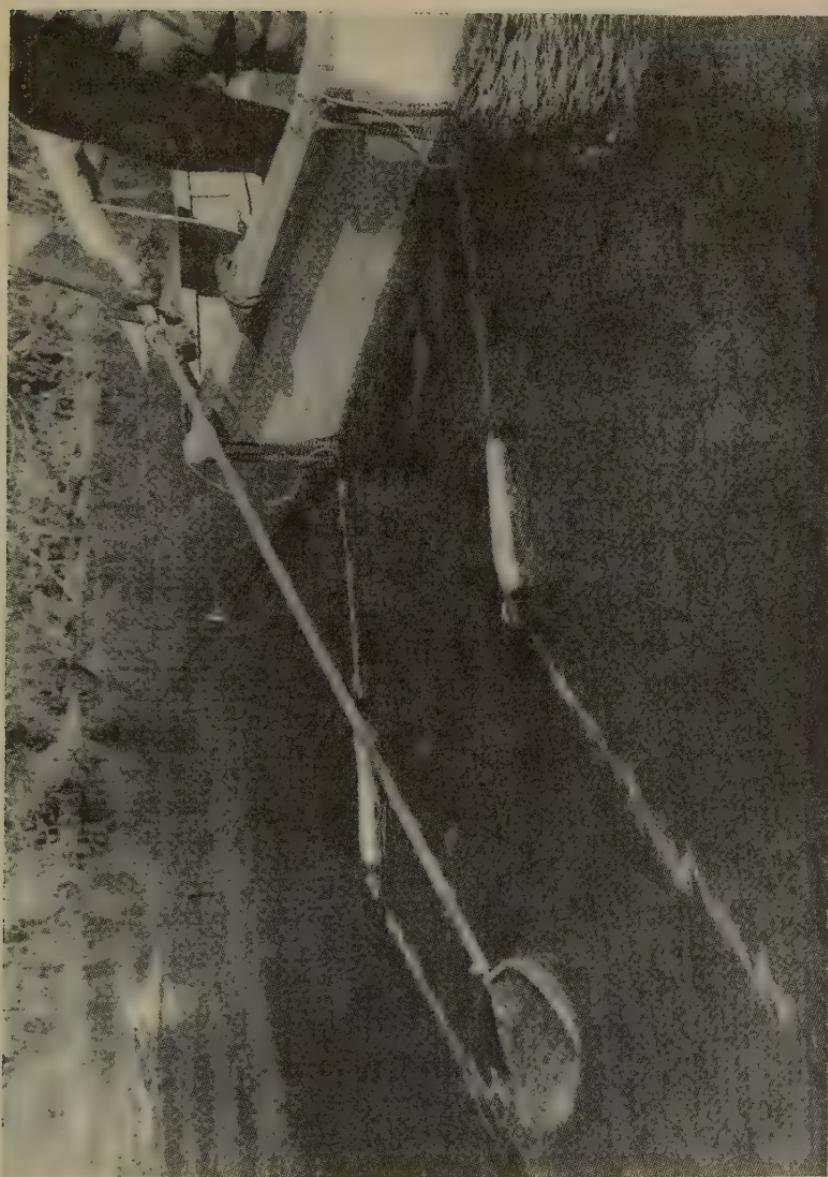
FIG. 4—The boat-mounted electrode system showing details of the dip-net and the attachment of the electrode supporting poles.
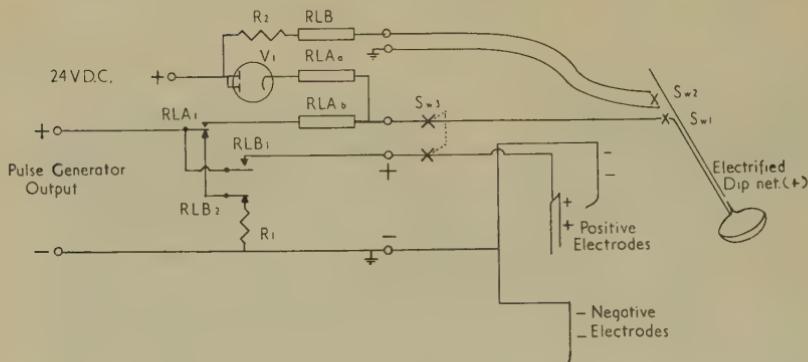
FIG. 5—Diagram of the control circuit for a boat-mounted electrode system. Components: R 1, load resistor adjusted to match the load presented by the fixed electrodes; R 2, series resistor to limit the current through the relay coil to the correct value; RLA and RLB, relays, Post Office type 3000 with 5 amp contacts, make and break; SW 1, and SW 2, spring release water-proof switches; SW 3, double pole single throw master switch (mounted on the boat handy to the second operator); V 1, Electron tube type 6X4.

Closing Sw1 operates the relay RLA, and electrifies the dip-net. If the dip-net is lifted out of the water, the pulse output is automatically disconnected from it as with the earth return system.

In use, a stop-net is placed at the downstream end of the section to be fished. Starting from the upstream end the boat is allowed to drift very slowly down stern-first guided by the second operator. In the shallower faster water it is necessary to have a third assistant downstream from the boat to collect those fish which are swept down out of the reach of the first operator.

It has been observed that fish are frightened by the weak fringe of the field, and by the passage of the boat; they attempt to find a way past. In order to maintain a high rate of catch it is essential that the electric field is effective *across the whole width of the stream.* The electrode system described does this with an electric current flow which is across the stream, between the two negative electrodes at the stream edges and the positive electrodes on the boat.

The aim is to produce sufficient voltage gradient *across the width of the fish to induce galanotaxis,* and turn it towards the positive electrode. The pulsatory direct current forces the fish to swim towards the positive electrode, and they are stunned within netting distance of the operator standing in the stern of the boat. The electrification of the dip-net also assists in the collection of the fish.

With this electrode arrangement the distribution of the electric current in the water is approximately spherical, and the voltage gradient is at a minimum midway between the positive and negative electrodes. The voltage gradient is proportional to the square of the distance between the electrodes.

As the power used is proportional to the square of the voltage, it is proportional to the fourth power of the electrode spacing for a given voltage gradient. Thus, for a given size of pulse generator the electrode spacing is critical.

Electrode voltages appropriate to the various water resistances are shown in Table 1. The voltage gradients produced are sufficient for the capture of trout of 3 inches and over in length.

TABLE 1—Output Voltages

Water Resistance ohms/in.^{3}	Output Voltage	
	Earth Return System	Boat Mounted Electrode System
5,000	300 volts	220 volts
10,000	400 volts	300 volts
20,000	600 volts	400 volts

This electrode arrangement is effective in streams of up to 20 ft wide and 4 ft deep. The voltage gradients produced in the water are higher when the resistivity of the stream bed material is greater than the resistivity of the water. If the resistivity of the stream bed material is significantly less than that of the water, then the field is distorted and weakened, and fish are let through.

Fish will pass round the ends of the negative electrodes with ease if the stream width exceeds the 20 ft span, and similarly they can pass underneath if the water depth is greater than 4 ft.

Sampling, by the mark and recapture technique, has given a catch rate of 47% for trout of 4 in. and over in length in a stream with a maximum depth of 4 ft and a maximum width of 20 ft.

The boat-mounted unit is potentially more dangerous than the earth-return system. It is recommended that operators gain experience with earth-return equipment before using the boat-mounted machine. An additional safeguard is the provision of an easily operated master switch (Sw3, Fig. 5) in a position handy to the second operator (i.e., the man controlling the boat).

THE PULSE GENERATOR

To meet the requirements set out, the pulse generator must provide output voltages of from 220 volts to 600 volts. With both electrode systems, the electrical resistance presented depends upon the water resistance, and the ground resistance. Measurements have been made in a variety of conditions, and the minimum value obtained for the earth return system was, electrical resistance of the electrode system (ohms) $= {}^1/_{29}$th of the specific

resistance of the water (ohms/inch³). Similarly, with the boat-mounted electrode system the electrical resistance (ohms) = $^1/_{200}$th of the specific resistance of the water (ohms/inch³).

Thus if the minimum water resistance is 5,000 ohms per cubic inch, the minimum resistance presented to the pulse generator by the boat mounted electrode system will be 25 ohms. At the specified output voltage of 220 volts the current is 9 amperes and the peak power is 1,900 watts. (The pulse power required increases as the water resistance decreases but not in direct proportion to the decrease in resistance).

The circuit diagram of the pulse generator used by the author is shown in Fig. 6. The power source is a gasoline engine driving an aircraft-type, high-frequency alternator. This is followed by a variable transformer, tapped for the appropriate output voltages. (With high-frequency alternating current both the alternator and the transformer are smaller than the equivalent low-frequency equipment). The A.C. is rectified to D.C. by thyratrons which are controlled by the pulse-forming multi-vibrator circuits. The D.C. is broken into pulsatory D.C. with a basic frequency of 2·5 pulses/sec and a duty cycle of 0·8. The 0·8 on time of this pulse is further broken up into 100 pulses per sec with a duty cycle of 0.33. The output of the pulse generator is fed through the control units already described.* The circuit shown is based on a 500 watt aircraft high-frequency alternator, and it will deliver a pulse of 250 volts on a load of 80 ohms. This is the lowest resistance encountered in a stream where the specific resistance of the water is 9,000 ohms/inch³, and the stream bed is of alluvial gravels. The machine cannot be used from the boat when the water has a lower specific resistance.

The rectifiers comprise 2 pairs of EN 32 Xenon thyratrons connected in parallel with suitable firing inductances. (In Fig. 6 single rectifiers are shown for simplicity.)

The electron tubes V1, V2 and V3, V4 comprise the two multi-vibrator circuits, the output of which appears across the resistors R6 and R7, and is thence connected directly to the thyratron control grids. The multi-vibrator frequencies can be altered by changing the capacitor pairs C2 and C3 or C4 and C5. The pulse length can be adjusted by altering the relative values of the resistor pairs R3 and R4 or R10 and R11.

A thermal delay switch (Sw3) is included in the circuit to ensure that the thyratrons are not switched on before the specified warming up time has elapsed. An instantaneous circuit breaker (Sw4) to operate at the peak permissible current is essential to protect the thyratrons in the event of an accidental short circuit.

V7, a zero-point anode, is desirable when the load is inductive, e.g., when there is a long length of cable coiled up. The condenser C8 (0·05 microfarad), was found to be necessary for the correct functioning of the multi-vibrator circuits. The meter M2 can be switched to measure:

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*In practice, the control unit of Fig. 5 is used for both types of electrode arrangement.
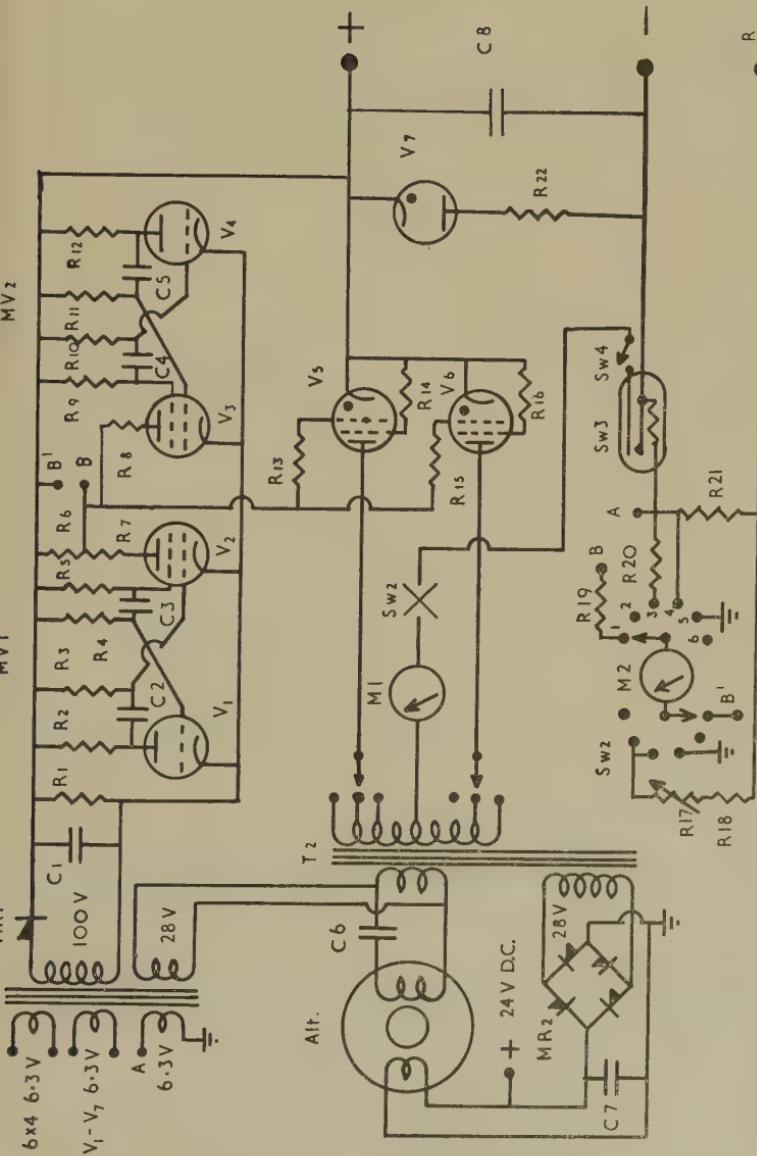
FIG. 6.—Circuit diagram of the pulse generator. Components: Alt, alternator, 500 watts at 1300 cycles; C1, 8 Microfarad, 450 volt electrolytic capacitor; C 2, 3, 0·01 microfarad, 800 volt A.C. capacitor; C 4, 5, 0·1 microfarad, 800 volt A.C. capacitor; C 6, power-factor correction capacitor; C7, 16 microfarad 450 volt electrolytic capacitor; C 8, *see text*; M 1, ammeter; M 2, 500 microampere meter with rectifier; MR 1, 125 volt metal rectifier; MR 2, 24 volt, 2 ampere metal rectifier; MV 1, high-frequency multi-vibrator; MV 2, low-frequency multi-vibrator; R 1, 10,000 ohms 2 watts; R 2, 5, 7, 8, 9, 12, 47,000 ohms 1 watt; R 3, 500,000 ohms 1 watt; R 4, 2,000,000 ohms 1 watt; R 6, 22,000 ohms 1 watt; R 10, 1,500,000 ohms 1 watt; R 11, 3,000,000 ohms 1 watt; R 13, 15, 470,000 ohms 1 watt; R 14, 16, 100,000 ohms 1 watt; R 17, 100,000 ohms variable resistance; R 18, 5,000 ohms 1 watt; R 19, meter shunt, 100 volts; R 20, meter shunt 10 volts; R 21, reference resistor – *see text*; SW 1, instantaneous circuit breaker; SW 2, two pole 6 position switch; SW 3, thermal delay switch; SW 4, on-off switch; T 1, flament and multi-vibrator supply transformer; T 2, variable centre-tap transformer; V 1, 4, 12AU7; V 2, 3, 6AU6; V 5, 6, EN 32 (*see text*); V 7, EN 32 (diode connected).

1. The multi-vibrator output. By noting the needle position; when the multi-vibrator is operating correctly regular checks will detect faults.

3. The filament voltage.

4 and 5. Resistance. The specific resistance of water can be measured by connecting a box such as is described by Haskell (1954) between the negative terminal and the terminal marked R. The value of the reference resistor R21 should equal the resistance of the box when filled with water with a specific resistance of 10,000 ohms/inch cube. Then the specific resistance of an unknown water =

$$10,000 \times \frac{\text{meter reading across box}}{\text{meter reading across reference}} \text{ (ohms/inch}^3\text{)}$$

The calculation can be simplified if, when the meter is switched to position 4, R17 is adjusted until the meter reads either $1/_{10}$th of full scale, or full scale, depending on whether the specific resistance of the water is greater than, or less than 10,000 ohms/inch cube. When the meter is switched to position 5, the specific resistance is the scale reading in tens of thousands, or thousands of ohms/inch^3 respectively.

Design Improvements

Recently developed silicon and germanium diodes are more efficient than the metal and electron tube rectifiers specified; e.g. it is an improvement to replace V 1 in Figs 2 and 5, by a silicon diode.

The size of the variable transformer (T2, Fig. 6) can be reduced if an auto-transformer, and bridge rectification by a pair of silicon diodes and a pair of thyratons, is used.

Another improvement would be the use of a 3-phase alternator.

Before a machine is designed, it is necessary to determine the specific resistance of the waters to be fished, and also to make up an electrode array and measure its electrical resistances in the deepest water of the streams to be fished.

Larger electrodes and a more powerful pulse generator will be needed to fish in water deeper than 4 ft. In streams more than 20 ft wide two or more boats could be used side by side to give an effective coverage.

ACKNOWLEDGMENTS

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OBSERVATIONS ON PHYTOPLANKTON ORGANISMS COLLECTED ON THE N.Z.O.I. PACIFIC CRUISE, SEPTEMBER 1958

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Summary

Phytoplankton was collected from an area of the Pacific Ocean between New Zealand and the Tonga and Fiji Islands during the New Zealand Oceanographic Institute Pacific Cruise in September 1958 on RNZFA *Tui*. Few previous records of phytoplankton in this region are available. Emphasis was placed upon the investigation of living organisms, except that the diatoms and armored dinoflagellates were reserved for later study in the laboratory. The majority of species that were observed belonged in the Coccolithophorineae and the unarmored dinoflagellates. There were seventeen species of unarmored dinoflagellates of which eleven are reported as new to science. Thirty-one species of Coccolithophorineae were identified. Both types of organisms were most abundant at station B67, east of Curtis Island in the Kermadec Island group. Other species of pelagic algae observed on the expedition belong in the following groups: Schizophyceae, Chlorophyceae, Euglenophyceae, Chrysophyceae, Xanthophyceae, and Cryptophyceae.

During September 1958, the New Zealand Oceanographic Institute sent an expedition of its members aboard the RNZFA *Tui* to the area of the Tonga Trench for the purpose of obtaining oceanographic data. The course of the *Tui* was north from Auckland, past the Kermadec Islands, into the Tonga Trench and to the Tonga Islands, thence west to the Fiji Islands and south to Wellington, New Zealand. It was the good fortune of the present author to accompany this group as far as the Fiji Islands for the purpose of collecting and making observations on samples of phytoplankton.

Samples were collected with a tow net of No. 25 mesh at each of the hydrological stations shown on the map (Fig. 1) and listed with pertinent data in Table 1. Part of each sample was observed in the living condition with a compound microscope. The remainder of each net sample was preserved in both 2% formaldehyde and a fixative containing 5 g iodine, 1 g potassium iodide, 24 c.c. formalin, 4 c.c. acetic acid, and 400 c.c. sea water. In addition, at most of the stations, a liter of surface water was filtered down to a 5 ml sample by millipore filters, and at many of the stations samples of phytoplankton in one liter of surface water were dried on millipore filter discs.†
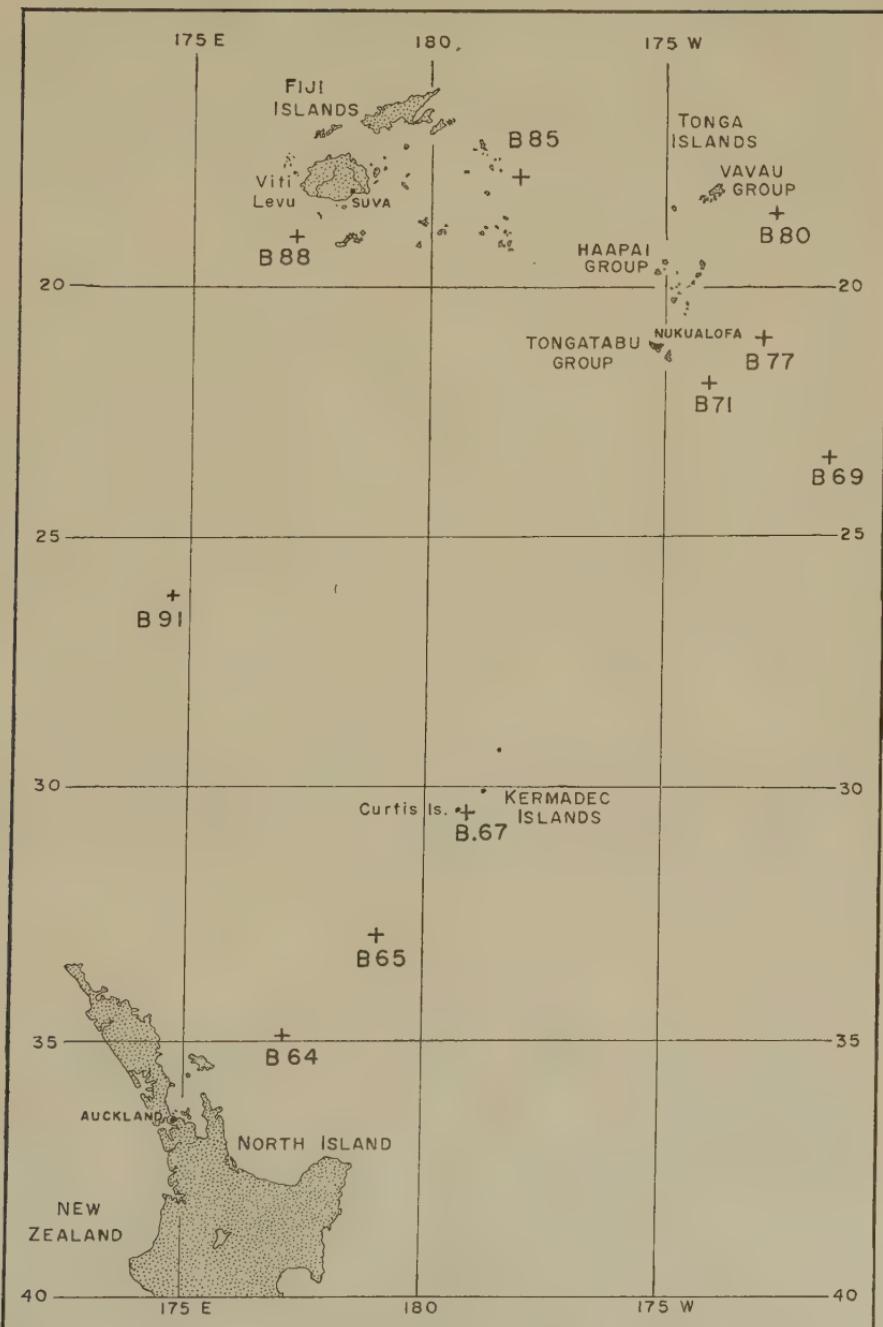
FIG. 1—Chart of the expedition indicating location of stations mentioned in the text.

TABLE 1—Data on Stations where Phytoplankton Specimens were Collected

N.Z.O.I. Station Number	Date	Latitude	Longitude	Temperature of Surface Water (°F)
B64	2-IX-58	34° 54′ S	177° 05′ E	60.0
B65	3-IX-58	32° 50.3′ S	179° 10′ E	59.3
B67	4-IX-58	30° 35.0′ S	179° 16′ W	62.2
B69	7-IX-58	23° 26.7′ S	171° 27′ W	71.8
B71	8-IX-58	21° 59′ S	174° 10′ W	72.3
B77	11-IX-58	21° 02′ S	172° 58′ W	72.7
B80	12-IX-58	18° 28′ S	172° 44′ W	78.3
B85	14-IX-58	17° 45′ S	178° 07′ W	78.4
*B88	17-IX-58	19° 03′ S	177° 13′ E	77.5
*B91	20-IX-58	26° 15′ S	174° 47′ E	70.0

*Specimens from stations B88 and B91 were seen only after fixation in formalin and iodine, or after drying on filter discs.

Because of the extreme difficulty, if not impossibility, of properly preserving most of these minute and fragile phytoplankton cells without damaging the protoplasmic membrane, flagella, and pigments, the observations contained in this report were made primarily on the sample populations in the living condition as they were collected. Supplementary information was obtained from preserved specimens when they were available. The list of Coccolithophorineae was made primarily from observations on specimens preserved in formalin or on dried millipore discs.

This report, which lists and describes all the phytoplankton collected except the diatoms and armored dinoflagellates, includes the Schizophyceae, Chlorophyceae, Xanthophyceae, Chrysophyceae, Euglenophyceae, Dinophyceae, and Cryptophyceae. Diatoms and armored dinoflagellates, common in most of the plankton samples, are being studied by Dr Vivienne Cassie at the New Zealand Oceanographic Institute. The system of classification proposed by Papenfuss (1955) is followed.

SCHIZOPHYCEAE

CHROOCOCCACEAE

Anacystis montana f. *minor* (Wille) Drouet et Daily 1952, p. 221. (Synonyms: ?*Coccochloris stagnina* Sprengel, ?*Microcystis litoralis* (Hansg.) Forti)

Specimens of a coccoid blue-green alga were found in the plankton at stations B69, B71, and B85 (Fig. 2). The diameter of the cells was 3 to 3·5 μ, and mature cells were spherical except before divisions, at which time they appeared slightly ellipsoidal. The cells contained a light blue-green pigment. Gas vacuoles were not present. The cells divided in three planes perpendicular to one another. They were arranged in compact colonies that measured up to 10·5 μ in diameter at station B69. The colonies at station B71 were somewhat larger than those at B69 with cells widely separated by the matrix material. At station B85 both compact and loosely organised colonies were found.

The exact identity of these specimens cannot now be determined with complete satisfaction. According to Drouet and Daily (1956), the specimen at hand was clasified as *Anacystis 'montana* f. *minor* Drouet et Daily. According to keys in Geitler (1932) the organism is very similar to *Microcystis litoralis* (Hansg.) Forti. This name is listed by Drouet and Daily as a probable synonym of *Coccochloris stagnina* Sprengel. However, the latter taxon includes only specimens with ellipsoidal to cylindrical cells, a shape which occurred in algae from my collections only before division.

OSCILLATORIACEAE

Oscillatoria thiebautii (Gomont) Geitler 1932, p. 967.

(Synonym: *Trichodesmium thiebautii* Gomont)

Loose bundles of parallel filaments of this species were observed in most of the collections. The cells were $1\frac{1}{2}$ to 2 times longer than broad (Fig. 4) and were of a light blue-green colour. The trichomes were surrounded by a thick layer of a watery mucilage which probably caused several filaments to remain united into bundles. The filaments could be separated from one another very easily, and isolated filaments were regularly seen. At station B69 the collections contained several filaments with a loose spiral as illustrated in Fig. 4. Since the dimensions and shape of the cells as well as the pigments of the spiralled filaments are the same as *O. thiebautii*, I have included them with this taxon.

Katagnymene spiralis Lemmermann 1899b, p. 354.

Large filaments of this species were observed at station B71. The diameter of the filament was 85 to 90 μ; that of the trichome was 17 to 28 μ. Hormogonia 17 to 20 μ in diameter were seen at station B69 (Fig. 3). No sheath was visible around the hormogonia, although the decomposed cells between them were clearly evident. The cells were of a light blue-green color.

MICROCHAETACEAE

Richelia intracellularis J. Schmidt *in* Ostenfeld and Schmidt, 1902, p. 146.

This organism was observed at stations B69, B71, B77, B85, and B88. Usually it was endophytic within *Rhizosolenia* spp, and *Hemiaulis hauckii,* but at stations B69, B77, and B85 it was observed also as an epiphyte on *Chaetoceros* spp. (Fig. 5). When growing as an epiphyte, the short filaments were attached to the spines of *Chaetoceros* at the intercellular spaces only. The trichome was attached at the end bearing the heterocyst and grew in a transverse direction around the diatoms. In most specimens the entire trichome of *Richelia* was tightly adherent to the walls of the diatoms. However, occasionally the narrower end of the trichome was free, and as a result the narrow sheath could easily be observed (Fig. 5). Karsten (1907, p. 403 and p. 536) also observed *Richelia* as an epiphyte on *Chaetoceros* in collections from the eastern Indian Ocean.
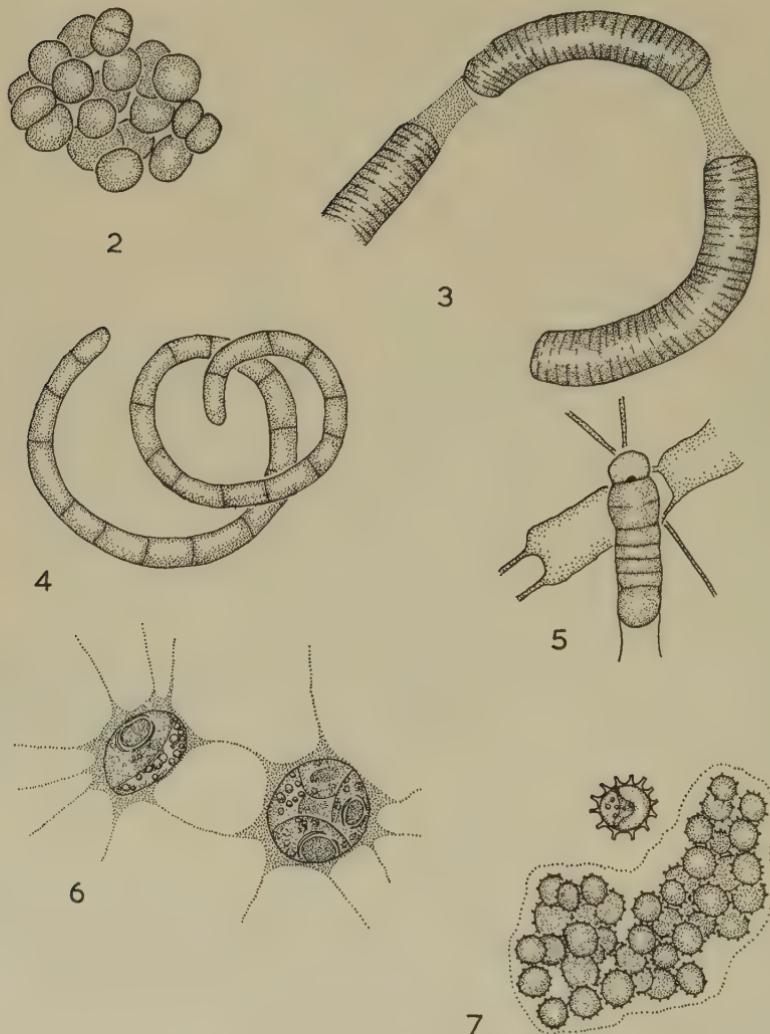
Fig. 2—*Anacystis montana* f. *minor.* × 2,000.   Fig. 3—*Katagnymene spiralis,* hormogonia. × 400.   Fig. 4—*Oscillatoria thiebautii.* × 600.   Fig. 5—*Richelia intracellularis, epiphytic* on *Chaetoceros.* × 1,000.   Fig. 6—Zooxanthellae within the protoplast of an Acantharian radiolarian. × 1,000.   Fig. 7—*Asterogloea undicola.* × 1,000.
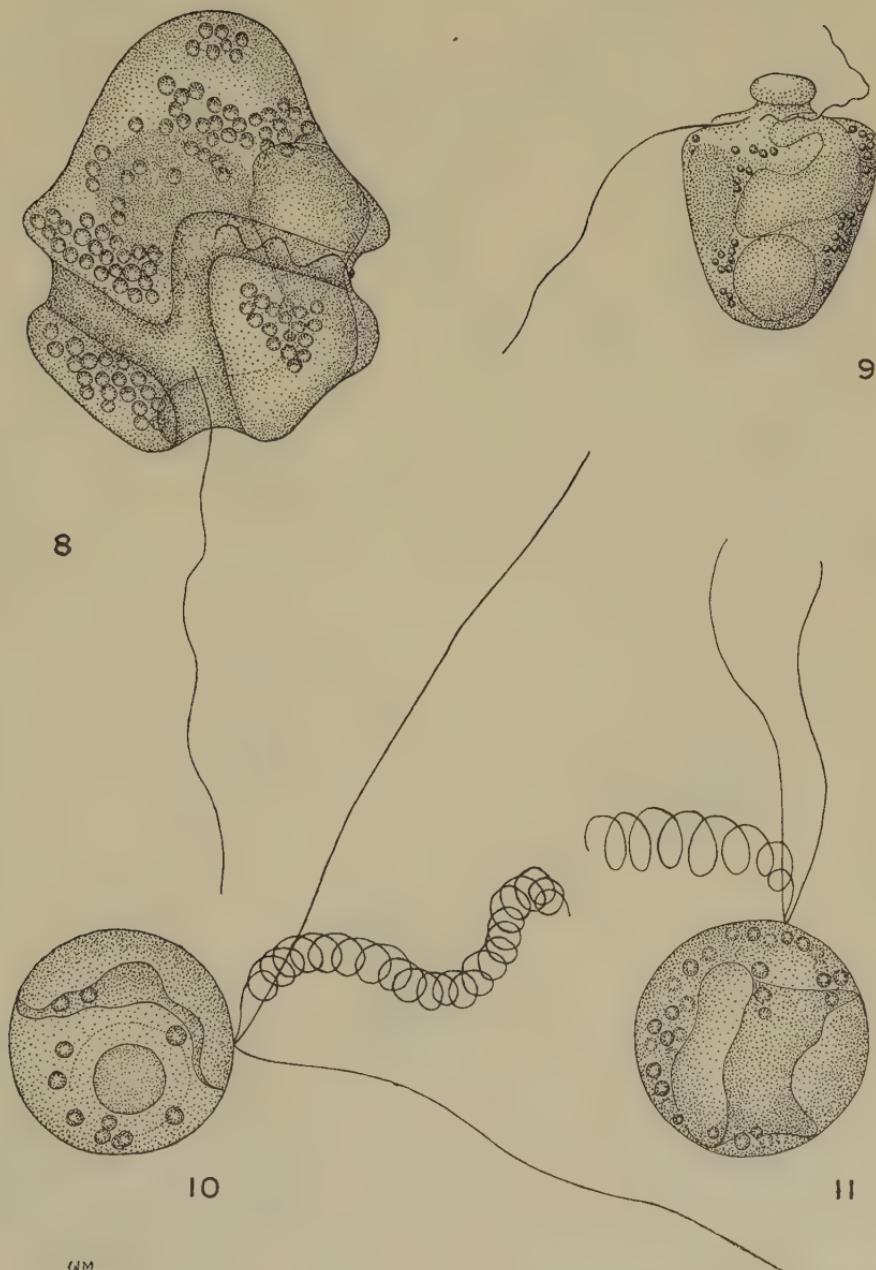
FIG. 8—*Gyrodinium kofoidii.* × 3,000.   FIG. 9—*Amphidinium lacustriforme.*
× 3,000.   FIG. 10—*Chrysochromulina* sp. from station B69. × 4,500.
FIG. 11—*Chrysochromulina* sp. from station B65. × 4,500.

12

a

b

c

d

13

14

15

16

17
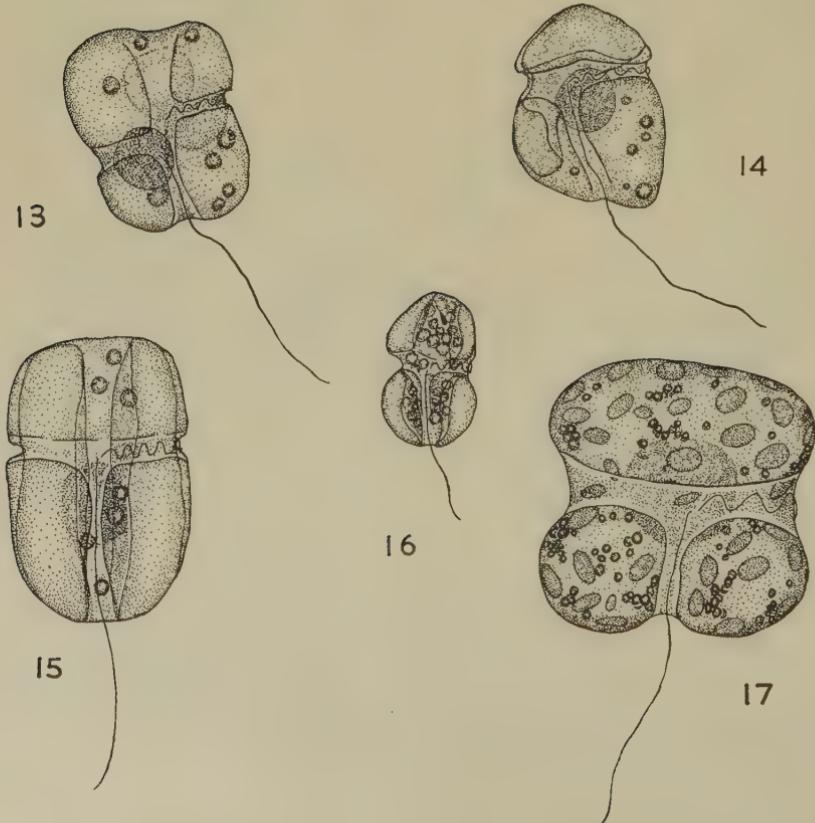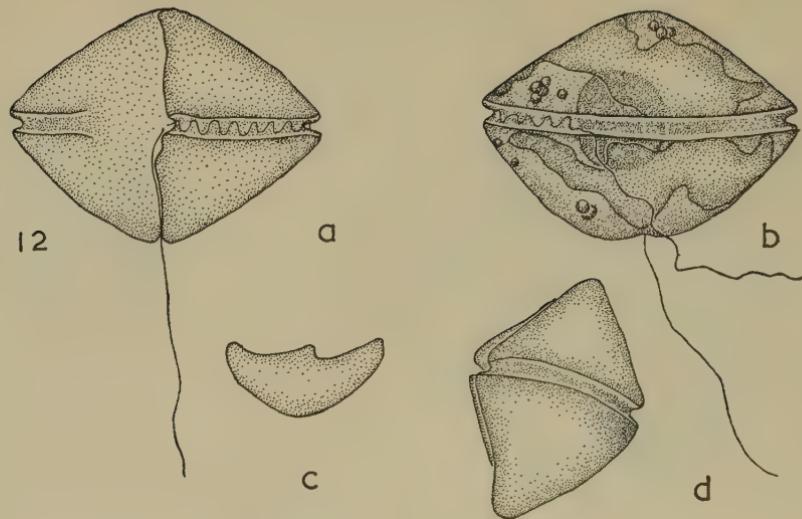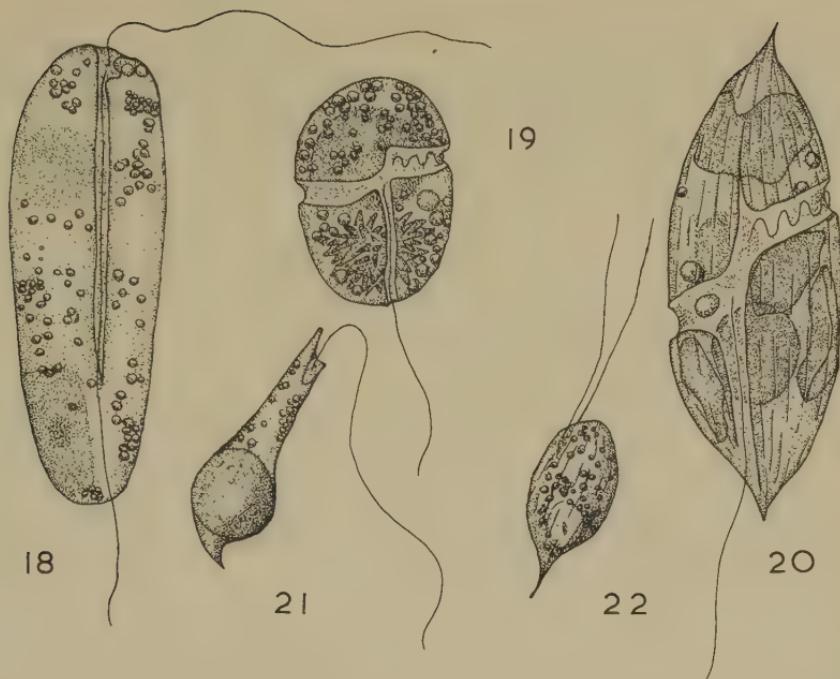
FIG. 18—*Protaspis tanyopsis.* × 2,000.   FIG. 19—*Gymnodinium cassiei.* × 2,000.
FIG. 20—*Gyrodinium chiasmonetrium.* × 2,000.   FIG. 21—*Euglenopsis zabra.*
× 1,500.   FIG. 22—*Chilomonas marina.* × 2,500.

FIG. 12—*Gymnodinium diamphidium.* a) ventral view; b) dorsal view; c) hypothetical
section of b; d) side view. c) × 1,000; all others × 2,000.   FIG. 13—*Gyro-
dinium phorkorium.* × 3,000.   FIG. 14—*Amphidinium aloxalocium.* × 2,000.
FIG. 15—*Gymnodinium simplex.* × 2,000.   FIG. 16—*Gymnodinium gram-
maticum.* × 2,300.   FIG. 17—*Gymnodinium exechegloutum.* × 2,000.

Sig.—12

The cells contained numerous granules, but no gas vacuoles were seen. The pigmentation of the cells of Richelia in its epiphytic habit appeared to be mostly phycoerythrin, giving the filaments a pinkish-grey appearance. The endophytic filaments were of a light blue-green color. Dimensions of the epiphytic filament in Fig. 5 were: length of filament 40 μ; heterocyst 6 μ by 5·2 μ; diameter of cell adjacent to heterocyst 7·5μ; diameter of terminal cell 6 μ.

CHLOROPHYCEAE

Palmellaceae

Pseudotetraspora marina Wille 1906, p. 20

Large green gelatinous sheets of *Pseudotetraspora marina* were common at station B67. The colonies in the net collections measured up to 1 cm in diameter. It is probable that the original colonies were somewhat larger, their delicate mucilate material having been torn in the process of their collection. The small green cells (Fig. 29) were 3 μ to 5 μ in diameter and were distributed throughout the mucilage in groups of two to four cells. The cells contained a brilliant green cup-shaped chromatophore that covered most of the peripheral area of the cell. Stellately lobed chromatophores as reported by Wille, were not seen in these specimens. Each cell contained a large and conspicuous pyrenoid located near the base of the chromatophore. An obvious sheath surrounding the pyrenoid was demonstrated to be starch by the iodine stain. Cells in different stages of division were observed and were similar to those described by Wille.

Fragments of colonies of *Pseudotetraspora marina* were found in the formalin preserved plankton samples. These were stained with haematoxylin and demonstrated a single small nucleus, approximately 1 μ in diameter, in each cell. The nucleus was usually located on the side of the cell opposite the pyrenoid. No motile cells were seen in this material, and none of the cells had pseudocilia.

XANTHOPHYCEAE

Pleurochloridaceae

Halosphaera viridis Schmitz 1879, p. 67.

One of the more common and conspicuous of the phytoplankton organisms collected on this expedition, *Halosphaera* was found at most stations between station B65 and B85. Usually it did not occur in the surface water, but specimens regularly could be found in the samples for which the net was allowed to sink one to two fathoms.

Cells of *Halosphaera* measuring up to 525 μ in diameter were seen. In many of the cells the disc-like chromatophores were aggregated into numerous groups, and as a result the larger cells closely resembled the general appearance of *Volvox* colonies. Several pyrenoids were usually present in each group of chromatophores. Such an arrangement of the protoplast probably is a stage in the development of zoospores or aplanaspores (see Dangeard, 1932). Unfortunately the mature spores were not seen.

Several cells were fixed in an iodine-formalin solution and stained with Mayer's acid haemalum. The protoplast did not stain properly because the stain did not easily penetrate the exceptionally thick cell wall. However, it was possible to observe several interesting features of the cell. The cell wall was clearly made up of two layers, an extremely thin outside layer that was stained by the haematoxylin solution, and a very thick $(2\,\mu)$ inner wall that remained unstained. Very small pits in the inner thick wall were observed (see Dangeard, 1932). The protoplast in the stained cells was multinucleate, each nucleus contained a large nucleolus. The chromatophores were small, disc-shaped to short ribbon-shaped. Numerous pyrenoids were seen, apparently free from the chromatophores. The distinct sheath which surrounded each pyrenoid gave no reaction with the iodine stain. Large globules of oil were present in most of the cells.

Asterogloea Pascher 1930, p. 420.

A. undicola sp. nov. (Fig. 7).

Cellulae globosae, junctae in inaequales colonias de 20–50 cellulis. Cellulae gelata hyalina circumdatae. Muri cellularum inaequaliter densati, formantes multas spinas, protrudentes et hebites, mensura aequales. Fines spinarum late truncati. 1 aut 2 chromatophores in omni cella, parietales et similes calicum, colore pallido-viridi. Linea media cellularum 3–5 μ.

Cells spherical, united into irregular colonies of 20 to 50 cells. Cells surrounded by a hyaline gelatinous substance. Walls of cells irregularly thickened to form numerous protruding blunt spines of regular size. The ends of the spines broadly truncate. One or two chromatophores in each cell, parietal and cup-shaped, of a light green color. Cells from 3 to 5 μ in diameter. One colony was observed entangled in the suspending protoplasmic mesh of a colonial radiolarian, *Sphaerozosma*. Collected at station B91.

This species closely resembles the only other known species of *Asterogloea*, the fresh-water *A. gelatinosa*. However, the cells of *A. undicola* are somewhat smaller and the spines are of a more distinctive type with the truncated ends. As with many of the Heterococcales, the form described here may be a stage in the life-history of other Xanthophyceae. There is need for careful analysis in culture of many species of the Heterococcales.

CHRYSOPHYCEAE

CHRYSOPHYCIDAE

CHRYSOCAPSACEAE

Tetrasporopsis Lemmermann 1899a, p. 103.

T. pelagica sp. nov. (Fig. 28).

Cellulae globosae-ovatae, cum membrana tenuissima, mersae in gelata aquosa formante parvam coloniam formae inaequalis in uno strato. Linea media coloniae fere 30 μ. Duo aurei chromatophores similes calicum aut vittarum in omni cellula aderant. Granulum, singulum et magnum, probabiliter leucosinum, inter chromatophores locatum est. Linea media cellularum 4–8 μ.

Cells spherical to ovoid, with a very thin membrane, embedded in a watery gelatinous substance forming a small monostromatic colony of irregular shape. Colony approximately 30 μ in diameter. Two cup-shaped or band-shaped golden chromatophores ware present in each cell. A single large granule, probably leucosin, was located between the chromatophores. Cells from 4 to 8 μ in diameter.

Zoospores which were observed within the gelatinous material, had a single flagellum approximately three times the length of the cell. They were regularly ovoid in shape and measured from 5 to 7 μ long and 3·5 to 6 μ wide. Each zoospore contained two chromatophores and a large leucosin granule in the center of the cell. Some contained two large granules at the anterior end of the cell. The zoospores were not observed swimming free of the gelatinous substance, but the flagella slowly moved within the gelatin. A stigma and contractile vacuoles were not seen. The colony observed was entangled between the spines of Acanthrometron in the sample at station B67.

PRYMNESIACEAE

Chrysochromulina Lackey 1939, p. 137.

Lackey described the genus, *Chrysochromulina*, to include a single new species that he found in a freshwater habitat in the United States. Parke, Manton, and Clarke (1955, 1956, 1958) described several new species of *Chrysochromulina* from marine environments near Great Britain. Parke *et al.* were able to determine that the third appendage of their species did not have the characteristic form or function of flagella. Rather, it was thinner, at times longer or shorter than the flagella, and seemed to function in attaching the cell. They called the appendage a haptonema. At times the haptonema was coiled close to the body of the cell. The cells of the marine species *Chrysochromulina* are ensheathed within a layer of very small scales that are visible only under extremely high magnification.

Because the previous records of marine *Chrysochromulina* were only from Great Britain, it was interesting that cells of *Chrysochromulina* were observed from two different localities on this expedition, stations B65 and B69. The cells at station B65 (Fig. 11) were spherical, 6 μ in diameter and with short flagella and a relatively long haptonema. (Unfortunately measurements of these appendages were not obtained.) The cells contained a single band-shaped golden chromatophore and many small granules.

At station B69 the species of *Chrysochromulina* (Fig. 10) was spherical, 6 μ in diameter, and had a peripheral band-shaped chromatophore with very light golden yellow pigmentation. The cell also contained a single large spherical body adjacent to one end of the chromatophore, probably a pyrenoid in the sense of Parke *et al.* Numerous granules were present in the cell. The flagella were approximately 20 μ long and the haptonema was approximately 30 μ long when fully extended.

W.M.

FIG. 23—*Amphidinium acutum* ×2500. FIG. 24—*Gymnodinium minor* ×1500.
FIG. 25—*Gymnodinium leptum* ×3125. FIG. 26—*Amphidinium micro-cephalum* ×2500. FIG. 27—*Gyrodinium apidiomorphum* ×2500. FIG. 28
—*Tetrasporopsis pelagica.* a) and b) Zoospores; c) a vegetative cell within the matrix. ×3125. FIG. 29—*Pseudotetraspora marina* ×3125.

Final determination of these species of *Chrysochromulina* must await more complete observations which should include details of the scales on the cells.

COCCOLITHOPHORINEAE

Table 2 includes those species of Coccolithophorineae, collected on the *Tui* expedition, which have characteristics distinctive enough to make their identification certain with only a light microscope. Unfortunately, many additional species in this group remained unidentified because an electron microscope was not available for this work.

SILICOFLAGELLATOPHYCIDAE

DICTYOCHACEAE

Dictyocha fibula Ehrenb. was common in most of the samples taken during the expedition. Living, uniflagellate cells of this species were seen at station B65. The skeleton of *Dictyocha octonaria* Ehrenb. was observed at station B67.

EUGLENOPHYCEAE

ASTASIACEAE

Euglenopsis Klebs 1893, p. 367.

E. zabra sp. nov. (Fig. 21).

Cellulae sine colore, similes cylindrorum cum posteriorem finem acutum. Finis proximus cum brevi et lato et tenui sulco ex quo singulum flagellum emersit. Gula parva; nullae striae in membrana cellulae. Longitudo cellularum 18–23 μ, latitudo 5–10 μ.

Cells colorless, cylindrical, usually with a more or less pointed posterior end. A wide shallow groove extending only a short distance was present at the anterior end of the cell. A single flagellum approximately as long as the cell emerged from the center of the groove. The gullet was small and very difficult to see. Striations were not evident on the periplast. The protoplast contained a large number of small granules and one very large golden granule that was probably an ingested food body. The nucleus was near the base of the gullet. The cells were only slightly metabolic and swam slowly, rotating and with the flagellum directed forward. Length of cell 18 to 23 μ, width 5 to 10 μ. Observed at station B67.

Despite the extreme difference in habitats, *E. zabra* closely resembles the fresh water species *E. vorax* Klebs, the only significant morphological difference lying in the pellicle which is smooth in *E. zabra* and striated in *E. vorax*.

Sig. 14

TABLE 2—Species of Coccolithophorineae and the Stations where They Occurred

Species	N.Z.O.I. Stations							
	B65	B67	B69	B71	B77	B80	B85	B91
Acanthoica acanthifera Lohm. 1912b, p. 240	x	x						
Acanthoica quattrospina Lohm. 1903, p. 68		x						
Acanthosolenia mediterranea Bernard, 1939, p. 40		x						
Anoplosolenia brasiliensis (Lohm.) Deflandre 1952, p. 458		x						
Anthosphaera robusta (Lohm.) Kamptner 1941, p. 86		x			x			
Calcidiscus quadriforatus Kamptner 1952, p. 235					x			
Calyptrosphaera insignis Schiller 1913, p. 604						x		
Coccolithus huxleyi (Lohm.) Kamptner 1943, p. 44		x	x		x	x		
Corisphaera fagei Bernard 1939, p. 37			x					
Cyclococcolithus leptoporus (Murr. et Blackm.) Kamptner 1954, p. 23	x	x	x		x			
Discosphaera tubifer (Murr. et Blackm.) Lohm. 1902, p. 141		x	x	x	x	x	x	
Gephyrocapsa oceanica Kamptner 1943, p. 45		x		x				
Helicosphaera carteri (Wallich) Kamptner 1954, p. 21		x						
Lohmannosphaera paucoscyphos Schiller 1925, p. 13		x						
Michaelsarsia splendens Lohm. 1912a, p. 40		x						
Ophiaster hydroideus (Lohm.) Gran 1911, p. 172	x	x				x		x
Pontosphaera caelamensis Lecal-Schlaeder 1951, p. 266		x					x	
Pontosphaera granii Gaarder 1954, p. 9		x						
Pontosphaera syracusana Lohm. 1902, p. 130					x			
Rhabdosphaera claviger Murr. et Blackm. 1898, p. 439		x			x	x		x
Rhabdosphaera stylifer Lohm. 1902, p. 143		x	x		x	x		
Scyphosphaera apsteini Lohm. 1902, p. 132		x			x	x		
Syracosphaera binodata Kamptner 1941, p. 82		x						
Syracosphaera corii Schiller 1925, p. 20		x						
Syracosphaera dalmatica Kamptner 1927, p. 178		x						
Syracosphaera histrica Kamptner 1941, p. 84		x						
Syracosphaera mediterranea Lohm. 1902, p. 134		x						
Syracosphaera molischi Schiller 1925, p. 21		x			x			
Syracosphaera pulchra Lohm. 1920, p. 134		x				x	x	
Thoracosphaera heimi (Lohm.) Kamptner 1944, p. 145		x					x	
Umbellosphaera irregularis Paasche in Markali and Paasche 1955, p. 97.							x	

DINOPHYCEAE

PROTASPIDACEAE

Protaspis Skuja 1939, p. 117.

P. tanyopsis sp. nov. (Fig. 18).

Cellulae elongatae, longitudo 28–30 μ, latitudo 9–11 μ; paulum compressae in fine proximo. Finis posterior late rotundus; finis proximus rotundus, etiam paulum truncatus. Cellulae non lentae et nullae rhizopodiae observatae sunt. Sulcus fere 2/3 longitudinem cellulae in longitudinem extendit. Margo sulci parvum lobum prope finem proximum cellulae protulit, et prope hac superficie sulcus in caveam tenuem abrupte patuit. Cellulae cum duobus flagellis, longitudine fere aequae. Ad finem proximum cellulae, ad acumen intra sulcum laxatum prope parvum lobum flagella fixa sunt. Unum flagellum semper ferme ad finem posteriorem cellulae dirigebatur et intra sulcum (in longitudinem) jacebat. Alterum flagellum ante dirigebatur. Cellulae sine colore.

Cells elongate, 28 to 30 μ long, 9 to 11 μ wide; slightly compressed in the anterior end. Posterior end broadly rounded, anterior end rounded to slightly truncate. Cells not metabolic and no rhizopodia were observed. A' groove extended longitudinally for approximately two-thirds the length of the cell. The margin of the groove produced a small lobe near the anterior end of the cell and near this area the groove broadened abruptly into a shallow cavity. Cells with two flagella, approximately equal in length. Flagella attached at the anterior end of the cell at a point inside the widened groove near the small lobe. One flagellum was usually directed toward the posterior end of the cell and lay within the longitudinal groove. The other flagellum was directed anteriorly. Cells not pigmented. A large nucleus was usually present in the anterior third of the cell. The protoplast was finely granular and contained numerous granules of a larger size. Large food vacuoles were usually present in the posterior part of the cell. Contractile vacuoles were not observed. Two small, highly refractive granules were regularly observed in the anterior end of the cell, usually in the area adjacent to the lobe of the furrow.

One individual was observed bearing four flagella, all directed anteriorly. Since there was evidence of the formation of a second longitudinal groove, it is assumed that the cell was in the process of dividing. The species was observed at station B67.

Protaspis tanyopsis is distinct from the four speices of *Protaspis* described by Skuja (1939, p. 117–119; 1948, p. 375) in that it is more slender than the other species of *Protaspis*. Also the anterior lobe on the groove in *P. tanyopsis* is not present in the previously described species. In cell shape *P. tanyopsis* resembles *P. obovata* Skuja 1948, p. 377 (also 1956, p. 354) more closely than any of the other three species of *Protaspis*. Both species are larger and more elongate than the other species and neither is markedly compressed. *P. obovata* differs from *P. tanyopsis* in that it produces a conspicuous rhizopodial system, and its cells are more rounded. *P. obovata* has been found only in fresh water in Sweden. The other species

described by Skuja occurred in the water of coastal regions of the eastern Balkan Sea. *P. tanyopsis* is the first species of this genus which has been shown to be distinctly marine and, indeed, truly pelagic.

When Skuja (1939, p. 117) founded the Protaspidaceae, he included it in the Euglenophyta. Upon later examination of the nucleus of *Protaspis obovata,* he observed that it had characteristics of the nuclei in dino-flagellates and removed the family to the Pyrrophyta in 1948. Huber-Pestalozzi (1955, p. 541–545) included the Protaspidaceae in the Eugleno-phyta. Both he and Skuja (1948, p. 375–376) reviewed the hypothesis that the Protaspidaceae may be an intermediate group between the Eugleno-phyta and the Pyrrophyta.

GYMNODINEACEAE

Amphidinium Claparède et Lachmann 1859, p. 410.

A. acutum Lohm. 1920, p. 140.

Cells that may be allied with this species were seen at station B67. In general form and size they closely resemble *Amphidinium acutum*; the chromatophores were, however, considerably different. In *A. acutum* there are two small golden chromatophores lying opposite one another in the anterior part of the hypocone. The cells that I observed contained two very large chromatophores almost as long as the hypocone (Fig. 23). They were cup-shaped at the broad end of the hypocone and tapered to a narrow point near the posterior end of the cell. They were placed close against the cell membrane and were of a light green colour. Despite this difference in pigmentation and size of the chromatophores, it seems best to include the cells observed under *A. acutum,* the taxon which it closely resembles in other characteristics. Length of cell, 17·5 μ; width 9·6 μ.

Amphidinium aloxalocium sp. nov. (Fig. 14).

Cellulae fere ovatae cum epivalva rotunda, acuta sed hebeti. Hypovalva fere 2 longior quam epivalva. Hypovalva longior in latere sinistro et, ad finem posteriorem, plana facienda. Zona praemediana, omnino cellulam circumdans, in aliquibus cellulis declinata 2–3 μ. Sulcus longitudinalis in epivalvam non extendit, longitudinem hypovalvae transvertens in linea obliqua ad sinistram. Cytoplasma minima granula cum nonnullis granulis magnis et refringere potentibus comprehendit. Singulus et magnus chroma-tophores similis coloris viridis oleae peripheriam epivalvae complevit, et secundus lobus chromatophorei in hypovalvam semper ferme extendit. Nucleus centraliter locatus semper ferme. Longitudo cellulae 12–14 μ lati-tudo 8–9 μ.

Cells approximately ovoid in shape, with a rounded epicone, bluntly pointed. Hypocone approximately twice the length of the epicone. The hypocone longer on the left side and becoming flattened toward the pos-terior end. Girdle premedian, completely encircling the cell, in some cells displaced by 2 to to 3 μ. Sulcus not extending into the epicone, traversing the length of the hypocone in a slant towards the left. Cytoplasm finely

granular, with several large refractive granules. A single large olive green chromatophore filled the periphery of the epicone and a second lobe of the chromatophore usually extended into the hypocone. Nucleus usually centrally located. Length of cell 12 to 14 μ, width 8 to 9 μ.

The slanted sulcus and the irregular shape of the hypocone in this small species of *Amphidinium* suggests a tendency for the cell to have torsion characteristic of many species of *Gyrodinium*. However, the torsion seems to be present only in the hypocone, since the trailing flagellum is attached at a point near to the girdle flagellum. Observed at station B67.

Amphidinium lacustriforme Schiller 1928, p. 132.

The flattened and much reduced epicone are characteristic of this species. The cells observed at station B69 (Fig. 9) agreed quite well with Schiller's description of the species except that they differed in not being compressed and contained only one band-shaped golden chromatophore rather than several chromatophores as in the cell illustrated by Schiller. Length of cell 10·5 μ, width 8 μ.

Amphidinium microcephalum sp. nov. (Fig. 26).

Cellulae ovatae, non compressae, cum minima et triangula epivalva. Zona et sulcus longitudinalis distinctus non aderant. Erat, autem, recessus cellulae ad acumen qua flagella fixa sunt. Flagellum zonae cellulam laxe circumdedit, et saepe finis non fixus ad finem posteriorem hypovalvae directus est. Trahens flagellum propinquum cellulae non jacuit sed semper ferme ab acumine alligandi tetendit. Protoplasma multos granulos, nucleum centraliter locatum, et in fine posteriori hypovalvae singulum et aureum chromatophorem similem vittae et prope contra unum latus cellulae jacentem, comprehendit. Pellicula erat macra et cum nullis signis et striis spectabilibus. Longitudo cellulae 9–12 μ, latitudo 7–8 μ.

Cells ovoid, not flattened, with a very small triangular epicone. Girdle groove and a distinct sulcus not present. There was, however, an indentation of the cell at the point where the flagella were attached. The girdle flagellum loosely encircled the cell and often the unattached end was directed toward the posterior end of the hypocone. The trailing flagellum usually angled away from the point of attachment. The protoplasm contained numerous granules, a centrally located nucleus, and in the posterior end of the hypocone, a single band-shaped chromatophore, golden in color, and lying close against one side of the cell. The pellicle was thin and with no visible markings or striations. Length of cell 9 to 12 μ, wth 7 to 8 μ. Observed at station B67.

Gymnodinium Stein 1878–83, p. 89–91. emend. Kofoid et Swezy 1921, p. 158.

Gymnodinium cassiei sp. nov. (Fig. 19).

Cellulae ovatae, non compressae, utrique fines late rotundi. Zona paulum praemediana, lata et alte impressa; suam latitudinem declinavit. Sulcus longitudinalis angustus extendens totam longitudinem hypovalvae. Protoplasma multos granulos et guttas olei mensurarum variantium comprehendit.

Nucleus magnus, plerumque intra et paene epivalvam complens. Chromatophores multorum et acutorum bracchiorum quae a media parte locata in hypovalva radiant; chromatophores de colore aureo. Longitudo cellularum 15–17 μ; latitudo 11–12 μ.

Cells ovate, not compressed, both ends broadly rounded. Girdle slightly pre-median, broad and deeply impressed; displaced its own width. Sulcus narrow, extending the entire length of the hypocone. Protoplast containing numerous granules and oil droplets of varying sizes. Nucleus large, mostly within and almost filling the epicone. Chromatophores golden, with numerous pointed arms that radiate from a center located in the hypocone. Cells 15 to 17 μ long, 11 to 12 μ wide. Observed at station B69.

This species is named for Mr and Mrs R. M. Cassie of the New Zealand Oceanographic Institute in recognition of their extensive work on the plankton of New Zealand.

Gymnodinium diamphidium sp. nov. (Fig. 12).

Cellulae bi-pyramidales, latiores quam sua longitudo, epivalva et hypovalva fere aequales spatio. Epivalva similior coni quam hypovalva in conspectibus ventralibus et dorsalibus. Latus dorsale convexum; latus ventrale concavum in latere dextro, latus sinistrum tendens esse planius et densius quam latus dextrum. Zona erat alta praeter dextrum latus ventrale prope sulcum longitudinalem qua minuebatur. Sulcus longitudinalis repraesentatus medio jugo in latere sinistro cellulae, jugum abrupte declinatum ad planum et dextrum latus cellulae. Jugum sulci longitudinalis totam longitudinem cellulae extendit sed ad utrosque fines cellulae altitudine minuebatur; finis posterior sulco longitudinali semper ferme scindebatur. Nucleus magnus centraliter locatus. Cytoplasma minima granula cum paucis maioribus granulis comprehendit. Singulus flavus chromatophores aderat, parietalis, et divisus in inaequalia segmenta simila taeniarum. Longitudo cellulae 15 μ, latitudo 20 μ; cellula fere 11 μ densa.

Cells bipyrimidal, wider than long, epicone and hypocone approximately equal in size. Epicone more conical than the hypocone in ventral and dorsal views. Dorsal side convex, ventral side concave on the right side with a tendency for the left side to be flatter and thicker than the right side (Fig. 12c). Girdle a deep furrow except on the right ventral side near the sulcus where it was diminished. Sulcus represented by a median ridge on the left side of the cell, the ridge abruptly declined to the right side of the cell. The sulcus ridge extended the entire length of the cell but diminished in height toward both ends of the cell; however the posterior end was usually cleft by the sulcus. Nucleus large and centrally located. Cytoplasm finely granular, with few larger granules. A single golden yellow chromatophore was present, parietal, and divided into irregular ribbon-shaped segments. Length of cell 15 μ, width 20 μ; cell approximately 11 μ thick. Observed at station B67.

G. diamphidium is very different from most species of the *Gymodinium-Gyrodinium* complex in the morphology of the ventral side. The sulcus is distinctive in being highly developed on only one side. *G. diamphidium* is similar to *G. conicum* Kofoid et Swezy (1921, p. 198) in the

characteristics of the sulcus. The shape of the cell and characters of the chromatophores, however, clearly separate these two species. An elaborate sulcus is also found in *Gyrodinium calyptroglyphe* Lebour (1925, p. 52) in which tongue-like processes overlap the sulcus groove. In this colorless species the sulcus is more prominent on the right-hand side rather than on the left as in *Gymnodinium diamphidium*.

Gymnodinium exechegloutum sp. nov. (Fig. 17).

Cellulae fere latiores sunt quam longiores, paulum compressae dorso-ventraliter. Epivalva ovata, latum latus proximum. Hypovalva ovata, finis posterior sulco longitudinali alte scissus. Zona sensim depressa, prominens in latere ventrali sed non manifesta in latere dorsali. Sulcus longitudinalis intra hypovalvam alte positus finem posteriorem cellulae similem lobi facit – lobus dextrus saepe maior quam sinistrus. Protoplasma erat in distinctis filis colligatis, cum minimis granulis et cum multis fasciculis granulorum, maiorum et sine colore. Chromatophores erant multi disci, parvi et ovati et de colore aureo, distributi intra fila colligata protoplasmae per peripheriam cellulae. Nucleus erat magnus, locatus prope mediam partem cellulae in latere dorsali. Magnum valuolum mediam superficiem cellulae complere videbatur. Longitudo cellularum 15–17 μ, latitudo 16–17·5 μ.

Cells approximately as broad as long, slightly flattened dorso-ventrally. Epicone ovate, the broad side anterior. Hypocone ovate, the posterior end deeply cleft by the sulcus. Girdle gradually depressed, prominent on the ventral side but not recognisable on the dorsal side. Sulcus deeply set within the hypocone, causing the posterior end of the cell to be lobed, the right lobe often larger than the left. The protoplast was in distinct strands, finely granular and with numerous clusters of larger colorless granules. The chromatophores were numerous small oval discs, of a golden color, distributed within the protoplasmic strands throughout the cell periphery. The nucleus was large, located near the center of the cell on the dorsal side. The central area of the cell seemed to be filled with a large vacuole. Length of cells from 15 to 17 μ, width 16 to 17·5 μ. Observed at station B69.

Gymnodinium exechegloutum resembles *G. dissimile* Kofoid et Swezy (1921, p. 204) in form but differs from it in possessing chromatophores and in the fact that the sulcus apparently does not extend into the epicone. Although it is similar to *G. gelbum* Kofoid (1931, p. 13), *G. exechegloutum* is much smaller and does not have a concave ventral side as does the former. *G. exechegloutum* may also be compared with *G. marinum* Kent 1880–81, p. 444. The two species differ considerably in comparative size of the hypocone and epicone as well as in the shape of the posterior end of the cell.

Gymnodinium grammaticum (Pouchet) Kofoid et Swezy 1921, p. 217.

Very small cells that were tentatively identified as *G. grammaticum* were found at station B69. The cells described by Pouchet (1887, p. 107) were from 25 to 26 μ long and approximately 18 μ in diameter. The cells that I observed (Fig. 16) were not as long (8·5 to 12 μ) and much narrower

(6 to 7 μ) than those described by Pouchet. The cells in my collection contained four parietal cup-shaped chromatophores of a light golden color, two in the epicone, and two in the hypocone. There were numerous refractive granules in the protoplast, but the nucleus was not observed. An elongate red stigma was present on the ventral side of the epicone. The stigma in the cells reported by Pouchet was larger than the ones I observed and lay in the hypocone parallel to the sulcus. Pouchet's description of *G. grammaticum* did not mention the chromatophores although a yellow color was attributed to the cell. It is possible that Pouchet's specimen was deteriorating and the chromatophores had lost their shape. The specimens that I observed seem closely allied to *G. grammaticum*, not only in general shape of the cell, but also in the presence of a stigma, which is unusual in marine species of dinoflagellates.

Gymnodinium leptum sp. nov. (Fig. 25)

Cellulae elongatae, angustae, paulum compressae dorsoventraliter. Epivalva similis rectanguli, finis proximus late rotundus. Hypovalva elongata, attenuans ad acumen obtusum ad finem posteriorem. Zona lata omnino cellulam circumdat. Sulcus longitudinalis, paulum obliquus ad sinistram, in latere sinistro hypovalvae acutae finitur. Protoplasma cum multis et magnis granulis, dense pressis; 3–5 chromatophores similes vittarum, breves et aurei, adesse sint. Nucleus non observatus est. Longitudo cellularum 12–15 μ, latitudo 5–6 μ.

Cells elongate, narrow, slightly flattened, dorso-ventrally. Epicone rectangular, the anterior end broadly rounded. Hypocone elongate, tapering to a blunt point at the posterior end. Girdle broad and completely encircling the cell. Sulcus slanted slightly to the left and terminating on the left side of the pointed hypocone. Protoplast with many densely packed large granules, three to five short, band-shaped golden chromatophores may be present. Nucleus not observed. Length of cells 12 to 15 μ, width 5 to 6 μ. This species was observed at station B67.

Gymnodinium leptum resembles *G. massarti* (Conrad) Schiller (1932, p. 382) in general form. The sulcus in *G. massarti* extends into the epicone, however, and slants to the right side of the hypocone rather than to the left side as in *G. leptum*.

Gymnodinium minor Lebour 1917, p. 192.

This species was observed at stations B65 and B67. The cells were almost spherical and measured approximately 33 μ, in both dimensions. The girdle of the cells observed did not become as narrow on the right side as in those described by Lebour. In other characteristics, however, there is a marked resemblance between the cells that I observed and the ones described by Lebour. The cells in my collections contained greenish-yellow spherical bodies (Fig. 24) similar to those described by Lebour as food bodies. It seems very likely that these bodies may be chromatophores since they were of a regular size and shape and showed no signs of being digested.

Gymnodinium simplex (Lohmann) Kofoid et Swezy 1921, p. 256.

Numbers of cells that can be assigned to this species were observed slowly moving about within a thin gelatinous material surrounding the spines of an Acantharian radiolarian. Zooxanthellae were abundant in the protoplast of the radiolarian (Fig. 6), but they contained a typical golden yellow pigmentation and pyrenoids, whereas the cells of *Gymnodinium simplex* had no pyrenoids and the chromatophores were yellow-green in color. Therefore it is unlikely that the dinoflagellate cells originated from the zooxanthellae.

This small species of Gymnodinium had broadly ellipsoidal cells with rounded ends (Fig. 15). The cells were not flattened and the girdle in the cells that I observed was distinct and well formed. Also the sulcus, although shallow, seemed to extend the entire length of the hypocone. The posterior flagellum was approximately twice the length of the hypocone. The protoplast contained four more or less cup-shaped chromatophores, two in the epicone and two in the hypocone. Some cells appeared to have only two chromatophores (Fig. 15) arranged lengthwise in the cell. These were probably in recently divided cells, and the chromatophores may have been about to divide. The nucleus was centrally located in the cell. The cytoplasm seemed quite clear, without much granular material. Length of cells 20 to 25 μ, width 11 to 12 μ. Observed at station B85.

Lohmann (1908, p. 265) described this taxon under a new genus, *Protodinium,* probably because he did not observe a sulcus groove on the cells. Kofoid and Swezy (1921, p. 256) assumed that the cells had a sulcus and transferred the species to *Gymnodinium*. Lebour (1925, p. 37) observed *Gymnodinium simplex* in culture at Plymouth and found a distinct sulcus, thus confirming the assumption of Kofoid and Swezy.

Gyrodinium Kofoid et Swezy 1921, p. 273.

G. apidiomorphum sp. nov. (Fig. 27)

Cellulae similes pirarum, non compressae aut solum compressae in latere ventrali. Epivalva similis dimidiae partis sphaerae, late rotunda, hypovalva elongata. fere 3 longior quam epivalva. Hypovalva attenuata ad acumen rotundum. Zona, declinata fere 6 μ, omnino circum cellulam in sinistra linea tortuosa extendit. Zona nonnihil ad dextram de acumine alligandi flagelli zonae extendit. Sulcus longitudinalis paulum obliquus ad sinistram indicans parvam summam torsionis esse in cellula. Cytoplasma minima granula comprehendit, massae cibi et globuli olei non visi. 10–12 chromatophores similes vittarum, parvi et inaequales et aurei, in peripheria cellulae aderant. Nucleus contraliter positus est. Longitudo cellulae 17–20 μ; latitudo 10–13 μ.

Cells pyriform, not flattened or only slightly flattened on the ventral side. Epicone hemispherical, broadly rounded. Hypocone elongate, approximately three times longer than the epicone, tapered to a rounded point. Girdle extended in a left hand spiral completely around the cell, displaced by approximately 6 μ. Girdle groove extended somewhat to the right of the point of attachment of the girdle flagellum. Sulcus slanted slightly to the left indicating a small amount of torsion in the cell. Cytoplasm finely

granular; food masses and oil droplets not seen. Ten to twelve small irregular band-shaped golden chromatophores present in the periphery of the cell. Nucleus centrally located. Length of cell 17 to 20 μ, width 10 to 13 μ. Observed at station B67.

Gyrodinium chiasmonetrium sp. nov. (Fig. 20).

Cellulae similes fusorum, utrique fines acuti, 2·5–3 longiores quam latitudo, fere rotundae in parte transversa. Summa cellulae cum striis longitudinalibus late separatis. Zona fere mediana, 3–4·5 μ declinata, fere sua latitudo. Sulcus longitudinalis ad posteriorem a dextra finem hypovalvae extentus et attenuatus ad acumen fere 10 μ intra epivalvam. Sulcus longitudinalis, probabiliter oriens e torsione in cellula, fere 5 μ ad dextram declinatus est. Nucleus prope mediam partem cellulae. Quattuor chromatophores pallidi-vivides, nonnihill similes vittarum, in periphera cellulae. Multa et parva granula sine colore aderant, item 1 aut 2 maiora et inaequalia granula cibi. Longitudo cellulae 30–33 μ, latitudo 11–12 μ.

Cells spindle shaped, pointed at both ends, 2·5 to 3 times longer than broad, nearly circular in cross-section. Surface of the cell with widely spaced longitudinal striations. Girdle approximately median, displaced from 3 to 4·5 μ (approximately its own width). Sulcus extended to the right posterior of the hypocone and tapered to a point approximately 10 μ within the epicone. The sulcus was displaced approximately 5 μ to the right probably as a result of torsion in the cell. Nucleus near the center of the cell. Four light-green chromatophores, more or less band-shaped, were located in the peripheral part of the cell. Numerous small colorless granules were present as well as one or two larger irregular food bodies. Length of cell 30 to 33 μ, width 11 to 12 μ. Observed at station B69.

Gyrodinium kofoidii sp. nov. (Fig. 8)

Cellulae angulares, compressae. Zona alte impressa, postmediana ad partem latissimam cellulae; zona fere 3 μ declinata. Sulcus longitudinalis latus et alte impressus; ad finem posteriorem hypovalvae ubi recessus altus effecit extendit. Epivalva longior quam hypovalva, late rotunda ad finem apicalem. Protoplasma cum fasciculis granulorum. Nucleus paulum premedianus. Unus longus chromatophores similis vittae in peripheria cellulae, plurimum comprehensus intra hypovalvam. Chromatophores colore viridi cum aureo. Longitudo cellulae 19–20 μ, latitudo 17–18 μ; crassitudo fere 10 μ.

Cells angular, compressed. Girdle deeply impressed, post-median, at the broadest part of the cell; girdle displaced approximately three microns. Sulcus broad and deeply impressed, extending to the posterior end of the hypocone where it caused a deep indentation. Epicone longer than the hypocone, broadly rounded at the apical end. Protoplast with clusters of granules. Nucleus slightly premedian. One long, band-shaped golden-green chromatophore in the periphery of the cell, mostly within the hypocone. Length 19 to 20 μ, width 17 to 18 μ, approximately 10 μ thick. Observed at station B67.

Gyrodinium phorkorium sp. nov. (Fig. 13)

Cellulae paene similes rectangulorum in conspectu ventrali, epivalva paulum latior quam hypovalva. Cellula rotunda in latere dorsali, paulum compressa in latere ventrali. Zona alte impressa et extendens omnino circum cellulam in sinistra linea tortuosa, 1–2 μ declinata. Sulcus longitudinalis in epivalvam extentus, latus ad finem praecedentem factus et levem lobum in epivalva efficiens. Sulcus longitudinalis longitudinem hypovalvae extendit et distinctum lobum in fine posteriori cellulae format. Protoplasma translucida chromatophores absunt. Nucleus prope mediam partem lateris ventralis, praesentia massarum magnarum cibi aliquando paulum declinatus. Cytoplasma granulorum minimorum nonnullos globulos olei sine colore comprehendit. Longitudo cellulae 12–15 μ, latitudo 8–10 μ.

Cells nearly rectangular in ventral view, the epicone slightly wider than the hypocone. Cell rounded on the dorsal side, slightly flattened on the ventral side. Girdle deeply impressed and extended completely around the cell in a left spiral, having a displacement of from 1 to 2 μ. Sulcus extended into the epicone, broadened at the anterior end and causing a slight lobing of the epicone. Sulcus extending the length of the hypocone and forming a distinct lobing of the posterior end of the cell. Protoplasm opaque, chromatophores absent. Nucleus near the centre of the ventral side, at times slightly displaced by the presence of large masses of food (Fig. 8). The cytoplasm was finely granular and contained several colorless globules of oil. Length of cell 12 to 15 μ, width 8 to 10 μ. Several cells were seen at station B67.

BLASTODINIACEAE

Paulsenella chaetoceratis (Paulsen) Chatton 1920, p. 320.

This interesting dinoflagellate is known only in its encysted form. It appears to be parasitic only upon species of *Chaetoceros*, attaching itself to the spines and producing a rhizoid that bores through the pores of the diatom wall. Several specimens of *Paulsenella chaetoceratis*, the only species of the genus, were observed on the spines of *Chaetoceros* in the net tow from station B67. The protoplasm contained a mass of colorless granules in addition to a large golden colored body present in the centers of most cells. The girdle groove was prominent in one specimen.

CRYPTOPHYCEAE

CRYPTOMONADACEAE

Chilomonas marina (Braarud) Halldal 1953, p. 58.

Cells of this species were numerous at stations B65 and B69. They differed slightly from the descriptions of Braarud and Halldal in having a more scattered arrangement of the trichocysts. In addition the periplasts of the cells that I observed were longitudinally striated (Fig. 22), a characteristic not previously reported for this species. The flagellate with an attenuated shape described by Lohmann (1920, p. 214) is very likely to be *Chilomonas marina.*

TABLE 3—List of Algae by Genera, and Hydrological Stations at Which They Were Found. (See Table 1 for locations of stations.) x = one species.

	N.Z.O.I. Stations								
	B65	B67	B69	B71	B77	B80	B85	B88	B91
Anacystis			x	x			x		
Oscillatoria			x	x	x	x			
Katagnymene			x						
Richelia			x	x	x		x	x	
*Pseudotetraspora		x							
Halosphaera	x	x	x	x	x	x	x		
Asterogloea									x
*Tetrasporopsis		x							
*Chrysochromulina	x		x						
Dictyocha	x	xx	x	x	x	x	x	x	x
*Euglenopsis		x							
*Protaspis		x							
*Amphidinium		xxx	x						
*Gymnodinium	x	xxx	xxx				x		
*Gyrodinium		xxx	x						
Paulsenella		x							
*Chilomonas	x		x						
Coccolithophorineae -- listed in Table 2.									

*Genera which are unrecognisable in the preserved samples of plankton.

DISCUSSION

Only limited conclusions concerning the general distribution of the phytoplankton can be drawn on the basis of this survey, because relatively few samples were examined in the vast area covered on this expedition. The algae occurring at the various hydrological stations are shown in Tables 2 and 3, which list Coccolithophorineae and the remaining algae respectively. From Table 3 it is evident that the blue-green algae occurred only in warmer waters, an observation that supports previous reports on pelagic blue-green algae. It is interesting that *Halosphaera, Dictyocha,* and several species of Coccolithophorineae were generally abundant at most of the stations (see Tables 2 and 3).

The phytoplankton population at station B67 is worthy of note because of the very large number of species and the comparatively greater abundance of individuals found there. Two groups of algae were especially prominent at station B67. Eleven of the seventeen species of unarmored dinoflagellates reported and twenty-six of the thirty-one species of Coccolithophorineae occurred at this station. Much further investigation with collections from many more stations as well as data on salinity, nitrogen, and phosphorus content of the waters is needed for the analysis of the factors controlling the unusually large phytoplankton population of this area.

This investigation on phytoplankton is one of the first in the Southern Hemisphere to include observations on living cells, many of which cannot be satisfactorily preserved. Sixteen of the thirty-one species of algae other than the Coccolithophorineae would not have been recognisable in the preserved condition. This evidence supports investigations on the small fragile species from other parts of the world that have shown that there are many species of these organisms, and that sometimes they may be the dominant members of the total phytoplankton population.

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CONSTITUENTS OF THE FLOWERS OF *METROSIDEROS EXCELSA* SOL. EX GAERTN

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Summary

Gallic acid, methyl gallate, ellagic, ursolic, and betulic acids have been isolated from the flowers of *Metrosideros excelsa*. The anthocyanins present have been identified as malvidin-3-monoglucoside and delphinidin-3-monoglucoside by chromatographic methods.

Introduction

Cooper (1958) has shown that considerable variation occurs in mass samples of *Metrosideros excelsa* (New Zealand "Pohutukawa") and *Metrosideros robusta* (New Zealand "Rata") collected from different localities in New Zealand and has suggested that the variation is due to introgressive hybridisation. Material collected from Mayor Island appeared to represent pohutukawa isolated from all other species of *Metrosideros* and growing under optimum conditions. Material collected from Westport represented rata growing in localities which are isolated from pohutukawa. As part of a taxonomic investigation of the constituents of rata, pohutukawa, and of hybrid species, flowers of specimens of *Metrosideros excelsa* collected in Auckland, where extensive variation can occur, have now been examined. Leaf samples of the trees from which the flowers were collected have been placed in the herbarium of the Auckland Institute and Museum under the numbers 69328 and 69329.

Previous examination of New Zealand *Metrosideros* species (family, Myrtaceae) has been confined to the constituents of the essential oils of a number of species. These appear to fall into two classes based on their chemical components (see Briggs, 1947), one class giving oils which contain mainly sesquiterpenes and sesquiterpene alcohols and the other which contains, in addition, significant proportions of terpenes and esters of terpene alcohols. Separation of the species on chemical grounds is not, however, paralleled by any corresponding botanical differences (Murray, 1949).

From an ether extract of the flowers, two polyphenols were isolated. The major phenol (0·67% yield), which could be separated by its solubility in sodium hydrogen carbonate, was identified as gallic acid while the second, present in minor amount (0·04% yield), was identified as methyl gallate. Both phenols were characterised by analysis and comparison (melting point, mixed melting point, ultraviolet spectrum, co-chromatography) with authentic samples. Further gallic acid was also isolated after hydrolysis of a methanol extract of the flowers. A third phenol, isolated directly from the methanol extract and also in greater amount (1·01%

total yield) after hydrolysis, was identified as ellagic acid by direct comparison with authentic material. In a survey of the systematic distribution of ellagic acid, Bate-Smith (1956) has shown that it occurs most consistently in the group Myrtinae (which includes the family Myrtaceae) of the order Myrtiflorae. It also invariably occurs in those families of the Dicotyledonae which contain gallates and gallotannins. Hathway (1957) has suggested that the incidence of ellagic acid may at least in part be related to the concomitant occurrence of gallate precursors. The co-occurrence of gallic acid, methyl gallate and ellagic acid in *Metrosideros excelsa* provides a further family for support of Hathway's view.

Triterpene acids have been isolated from the bark and heartwood of a number of species of the Myrtaceae including the barks of the New Zealand species, *Leptospermum scoparium* (Corbett and McDowall, 1958) and *Leptospermum ericoides* (Corbett and McCraw, 1959). Triterpene acids have now been found to occur in *Metrosideros excelsa* and those from the flowers were readily isolated from the ether extract by chromatography on silica gel following the procedure of Corbett and McCraw. The acid mixture consisted mainly of a compound identified as ursolic acid (1·1% yield) by comparison with an authentic sample and by the preparation of derivatives. By further chromatography after the separation of ursolic acid a second acid was isolated and identified as betulic acid (0·01% yield).

The ether extract contained only minor amounts of neutral constituents identified as long chain aliphatic esters by infrared spectroscopy. These were not readily purified by chromatography on alumina and were not further investigated.

The anthocyanins responsible for the dark crimson colour of the flowers were initially examined by the scheme of Robinson and Robinson (1931) involving colour reactions and solvent distribution studies, which indicated that the principal compound was malvidin-3-monoglucoside (oenin). Paper co-chromatographic examination utilising the methods summarised by Harborne (1958) and using a large number of authentic anthocyans confirmed the presence of oenin and showed the presence in lesser amount of delphinidin-3-monoglucoside (Table 1).

Table 1—R_F Values of Pohutukawa Anthocyans

Glycoside	BAW*	1%HCl*	HAc–* HCl	Aglycone	Forestal*
Pigment 1	0.39	0.03	0.18	Anthocyanidin 1	0.30
Pigment 2	0.26	0.06	0.30	Anthocyanidin 2	0.60
Delphinidin-3-glucoside	0.39	0.03	0.18	Delphinidin	0.30
Malvidin-3-glucoside	0.26	0.06	0.30	Malvidin	0.60

*Solvent systems are described in the experimental section.

Experimental

Analyses are by Dr A. D. Campbell, University of Otago, New Zealand. Infrared spectra were measured as KBr discs and ultraviolet spectra were measured for EtOH solutions. Descending paper chromatography of phenolic

constituents was carried out on Whatman No. 1 paper with the following solvent systems: (BAW) butan-1-ol—acetic acid—water (4 : 1 : 5); (5% HAc) 5% aqueous acetic acid; (1% HCl) water—12N hydrochloric acid (97 : 3); (HAc–HCl) water—acetic acid—12N hydrochloric acid (82 : 15 : 3); (Forestal) acetic acid—conc. hydrochloric acid—water (30 : 3 : 10).

EXTRACTION OF *Metrosideros excelsa* FLOWERS

Fresh flowers (dry weight, 535 g) collected from Mechanics Bay, Auckland, were air-dried and extracted (Soxhlet) with ether and again with methanol. Preliminary trial extraction with light petroleum had given a small amount of waxy oil which was added to the ether extract. The ether extract on concentration gave successive crops of amorphous triterpene acids which were washed with a little cold ether and extracted with hot water. The ether washings were returned to the ether extract, the final ether concentrate extracted with hot water (3 × 50 cc) and the combined aqueous extracts continuously extracted with ether for 40 hr. The ether solution was extracted with 10% aqueous sodium hydrogen carbonate, dried, and evaporated to an oil (A).

GALLIC ACID

The sodium hydrogen carbonate extract was acidified and re-extracted with ether. Removal of solvent and repeated crystallisation of the residue from water (charcoal) gave gallic acid (3·65 g) as needles, m.p. and mixed m.p. 238°–240° (decomp.) raised to 256°–258° on drying, *in vacuo*, at 125° [Found: C, 48·9; H, 3·3. Calc. for $C_7H_6O_5$: C, 49·4; H. 3·6%] $\lambda_{max.}$ 272 m μ (log ε 4·04). The natural and authentic material had identical R_F values; 0·50 (5% HAc), 0·72 (BAW). Sublimation, *in vacuo*, at 250°–270° gave pyrogallol, m.p. and mixed m.p. 132° (decomp.). Tri-O-methyl-gallic acid had m.p. and mixed m.p. 168°–169°.

METHYL GALLATE

Purification of the oil (A) by Jurd's method (1956) and repeated crystallisation of the phenol from water gave methyl gallate (216 mg), needles, m.p. 198°–200° (decomp.) undepressed by a synthetic sample prepared by Shöpf and Winterhalder's method (1940) [Found: C, 52·1; H, 4·6. Calc. for $C_8H_8O_5$: C, 52·2; H, 4·4%] $\lambda_{max.}$ 217 m μ (log ε 4·70) and 276 m μ (log ε 4·30). The natural and synthetic material had identical R_F values; 0·54 (5% HAc), 0·86 (BAW).

URSOLIC ACID

The crude triterpene acids, which gave strong Liebermann-Burchard reactions, were re-extracted with ether, the extract concentrated and treated with 20% aqueous sodium hydroxide (5 × 100 cc). The precipitated sodium salts were dried, dissolved in methanol, acidified, and the amorphous acids purified by chromatography in benzene on silica gel. Fractions eluted with benzene-ether (9 : 1) gave ursolic acid (5·85 g), needles (from ethanol),

m.p. and mixed m.p. 285°–286° (decomp.), $[\alpha]_D^{20}$ + 65·8 (c 1·2 in pyridine), $[\alpha]_D^{20}$ + 69·7 (c 0·6 in CHCl$_3$) [Found: C, 78·7; H, 10·4, Calc. for C$_{30}$H$_{48}$O$_3$: C, 78·9; H, 10·6%]. The infrared spectrum was identical with that of an authentic sample. The acetate had m.p. and mixed m.p. 282°–285° (identical infrared spectrum) and the methyl ester, prepared by the action of diazomethane, had m.p. 170°.

BETULIC ACID

Residues obtained from the mother liquors of ursolic acid were rechromatographed on silica gel. Fractions eluted with benzene-ether (10 : 1), after separation of further ursolic acid, gave betulic acid (82 mg), needles (from ethanol), m.p. 310°–312°, undepressed by an authentic sample. $[\alpha]_D^{20}$ + 7·9 (c 0·4 in pyridine) [Found: C, 78.5, H, 10.5. Calc. for C$_{30}$H$_{48}$O$_3$: C, 78·9; H, 10·6%]. The infrared spectrum was identical with that of an authentic sample.

ELLAGIC ACID

The methanol extract was concentrated to dryness, *in vacuo*, extracted with hot water (2 × 500 cc) and the insoluble residue crystallised from pyridine to yield ellagic acid (156 mg), m.p. > 360°. The aqueous solution was hydrolysed by addition of concentrated sulphuric acid to form a 3% aqueous solution followed by heating under reflux at 100° for 3 hr. The solution was decanted from a black insoluble tar and extracted with ether (8 × 250 cc) to give further gallic acid, m.p. and mixed m.p. 240°–244°. Further extraction of the hydrolysate with ethyl acetate (8 × 250 cc) and concentration of the dried extract gave ellagic acid (3·7 g), yellow needles, m.p. > 360°, from pyridine [Found: C, 56.0, 55·4; H, 2·5, 2·6. Calc. for C$_{14}$H$_6$O$_8$: C, 55·6; H, 2·0%] $\lambda_{max.}$ 255 mμ (log ε 4·68) and 367 mμ (log ε 4·02), R$_F$ 0·33 (BAW), 0·32 (Forestal). Ellagic acid gave the usual colour reactions and the infrared spectrum was identical with that of authentic sample crystallised from the same solvent. Ellagic acid tetraacetate, needles from acetic anhydride, had m.p. and mixed m.p. 341°–343°.

Glucose was identified in the aqueous hydrolysate by circular co-chromatography with authentic material in phenol-water (9 : 1).

ANTHOCYANINS

A cold 1% aqueous hydrochloric acid extract of fresh flowers was purified and examined for anthocyanins by Robinson and Robinson's method and also by descending paper chromatography in three solvent systems. Accurate R$_F$ values, the average of six determinations, were only obtained after the pigments had been purified by banding on to Whatman No. 3 paper and eluting the bands with methanol-hydrochloric acid. Results for anthocyanins and anthocyanidins, obtained after hydrolysis, are summarised in Table 1. Glucose was identified in the hydrolysate of each pigment by circular co-chromatography.

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CORROSION OF COPPER AND COPPER ALLOY APPLIANCES IN NEW ZEALAND COPPER HOT-WATER CYLINDERS

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Summary

Of some thirty rapid hot-water cylinder failures examined, all were associated either with dezincification of alpha/beta brass brazed seams or localised attack beneath corrosion product or other deposits. No failures due to corrosion of silver brazing alloy or galvanic interaction between silver brazing alloy and copper were seen.

INTRODUCTION

The present work is part of an investigation into the corrosion behaviour of untinned copper and copper-alloy water appliances in New Zealand. Causes of failure of hot-water cylinders investigated by Dominion Laboratory over the last twenty years can be broadly classified into several types—

1. Dezincification of brazed seams. Many of the earliest cylinders manufactured in New Zealand were fabricated using 60/40 brass spelter and these appear to have failed with monotonous regularity, particularly in certain districts.

2. A number of cases of pitting of hot-water cylinders appeared to be associated with waters of low pH and high carbon dioxide content, but these cases are not well documented and may in fact have been cases of under-deposit attack.

3. Invercargill water: Until 1959, when a new water supply was brought into use, the Invercargill water supply (untreated) consisted of low pH, high carbon dioxide artesian water contaminated by sea water. This mixture was extremely corrosive and the average life of copper hot-water cylinders in which no alpha/beta brass brazing had been used was about two years.

EXPERIMENTAL

A number of hot-water cylinders and other appliances, some of which had failed in service, were examined visually and by metallographic sectioning.

RESULTS

In the case of hot-water cylinder seams brazed with 60/40 brass, initial dezincification of the beta phase was followed by dezincification of the alpha phase, and a spongy mass of copper remained. According to a number of manufacturers and plumbers, dezincification troubles are by no means universal, and in many districts hot-water cylinders brazed with 60/40 brass give satisfactory service. It is also asserted that such variables as brazing temperature and type of "bronze" rod affect the lives of "bronze-welded" cylinders very critically, but it has not yet been possible to investigate these claims. There was some evidence that corrosion products from corroding seams could, by falling on to copper at lower points of cylinders, initiate pitting. Vertical lines of pits, mainly on the top surfaces of corrugations, were encountered a number of times. Pits on the bottoms of cylinders brazed with brass could presumably also have been caused in this manner.

One Lower Hutt cylinder had failed by pitting of the bottom beneath particles of earth, apparently introduced early in the service life. The cylinder in question had been fabricated with "Silbralloy" brazing rod and the water supply had been lime-treated to raise pH. A similar failure occurred in a copper hot-water pipe at Thames – the water supply concerned being quite uncorrosive.

Failures, in raw Invercargill water, of cylinders brazed with "Silbralloy" were characterised by vertical streamers of green crystals (basic sulphate) under which severe uniform corrosion of copper had occurred. Unattacked surfaces were almost black. Failures often appeared to be associated with the vertical brazed seam but in such cases further examination revealed that failure was also imminent under other corrosion product streamers remote from the brazed seam.

For some months before the new supply came into use, Invercargill water was treated with caustic soda and (for part of the time) "Calgon". The corrosion of hot-water cylinders was greatly reduced. Test cylinders examined after about a year in the treated water showed no signs of corrosion product streamers or of the black material previously seen, and were in excellent condition.

Crevice corrosion, though observed in a few instances, had no significant effect on any cylinder failure.

Examination of metallographic sections from hot-water cylinders, other appliances and test assemblies after exposure in various hot and cold New Zealand waters indicated that alpha/beta brass, whether in contact with copper or not, always deteriorated more rapidly than copper, silver brazing alloys, gunmetals, or any combinations of these latter alloys. Mild galvanic corrosion of copper adjacent to "Easyflo", "Silbralloy" and "Silfos" was observed in a number of cases (Fig. 1) but in other instances there was no special attack of copper at the bimetallic junctions. Cases were also seen where general even attack of both copper and silver brazing alloy had occurred (Fig. 2) and where the silver brazing alloy was more heavily attacked than the copper (Figs 3, 4). Thus the "Silfos"/copper and

FIG. 1—Preferential attack on copper adjacent to "Silfos" in a hot-water cylinder after six years' service in lime-treated Hutt water. (pH 8–9, Hardness 80–100 ppm, Cl⁻ 20 ppm). × 75. Ammonium persulphate etch.

FIG. 2—Attack on both "Silbralloy" and copper at a hot-water cylinder braze after two years in untreated Invercargill water (pH 6·2–6·4, hardness 125 ppm, CO_2 17 ppm, Cl⁻ 130 ppm, SO_4 = 50 ppm). × 80. Ammonium persulphate etch.
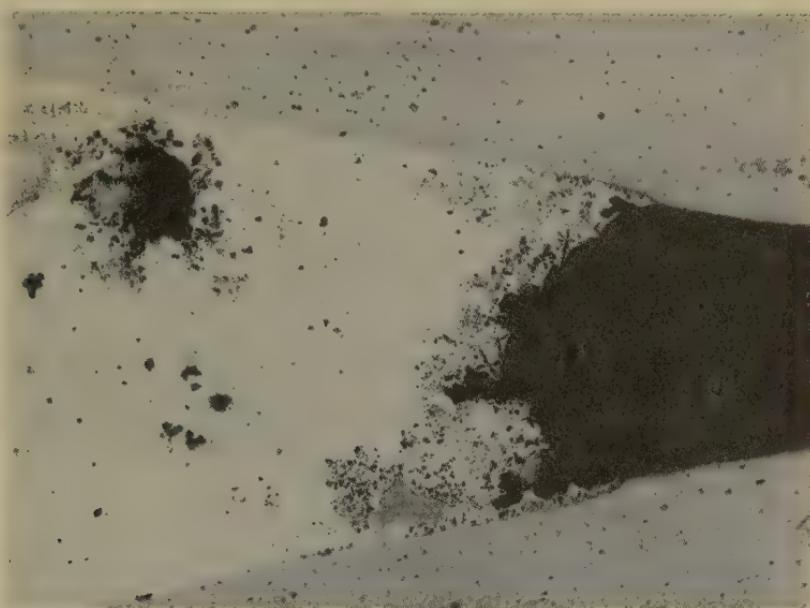
FIG. 3—Preferential attack on "Easyflo 2" adjacent to copper in a test assembly exposed for six months to hot untreated Invercargill water. Note selective attack of dendritic constituent. Copper particle pressed into porous corroded portion of braze metal during polishing demonstrates soft spongy nature of remaining material. × 115. Unetched:
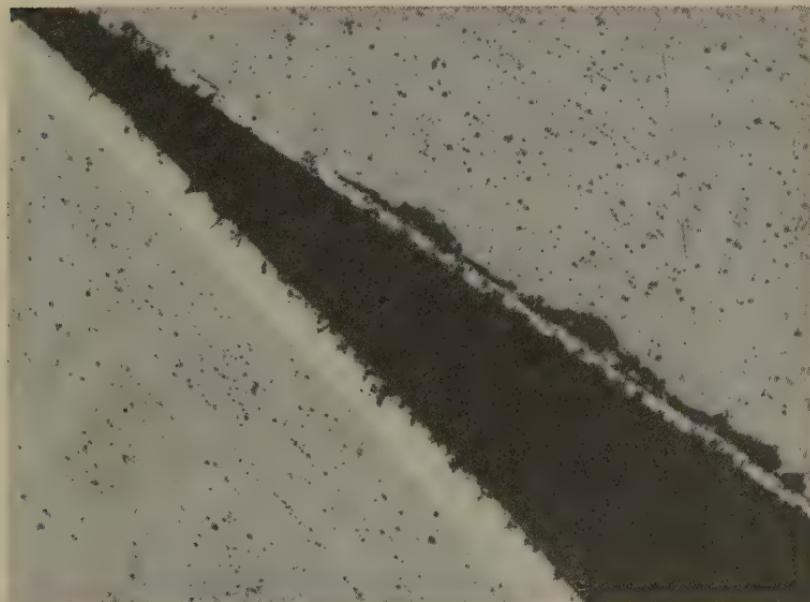
FIG. 4—A different portion of the specimen shown in Fig. 3. Selective attack on dendritic constituent is again apparent. Note also attack on copper beneath "Easyflo 2" with all the dendrites corroded away. × 115. Unetched.

"Silbralloy"/copper couples were not very active, copper being the anodic member of the couples, and the "Easyflo"/copper couple was rather more active and of variable polarity. Laboratory measurements confirmed this rating. One example of an alpha/beta brass/"Easyflo" junction displayed no galvanic corrosion; neither did a copper/"Silfos" braze exposed to intermittent cold sea water immersion and salt spray for twelve years.

For any particular couple, no systematic differences of polarity were observed between junctions in crevices, junctions in easily accessible places, and junctions under corrosion product deposits. Dezincification of alpha/beta brass appeared to be more severe in seams than in open locations, but no measure of the difference was obtained.

The corrosion mechanisms of the silver brazing alloys are of interest. "Easyflo" typically has a cast structure as shown in Fig. 5. In all cases seen, the yellow, copper-rich dendrites (Rudolph and Wagner, 1956) were

FIG. 5—A sound region of the specimen shown in Fig. 3, illustrating metallographic structure. In the unetched condition the dark dendrites are light yellow on an almost white background. × 470. FeCl₃ etch.

preferentially attacked, and while this phase was present the alloy was anodic to copper (Fig. 3). The remaining silver-rich matrix however was cathodic to copper (Fig. 4) so the polarity of "Easyflo" with respect to copper was not fixed, but reversed as corrosion progressed. "Silfos" and "Silbralloy" were never anodic to copper, according to micro-sections of corroded junctions. The relative polarities of the individual phases making up these alloys varied however, usually the copper-rich phase was preferentially attacked (Fig. 6) but cases were seen in both "Silfos" and "Sil-

bralloy'' where the Cu_3P phase (blue under the microscope) was preferentially attacked (Fig. 7). In one case examples of both types of corrosion were obtained from the same hot-water cylinder.

FIG. 6—"Silbralloy" after ten months' exposure in an Invercargill hot-water cylinder. Preferential attack on the copper-coloured phase (lighter in photomicrograph). × 1,300. Unetched.

DISCUSSION

The difference in behaviour between seams brazed with silver brazing alloys and alpha/beta brass is influenced by several factors. Alpha/beta brass is, of course, less noble than copper, while the silver brazing alloys are (according to metallographic examination) more noble than copper, so one might expect in the former case, severe corrosion due to a 'small anode-large cathode' effect while with the silver brazing alloys, 'large anode-small cathode' would not have such disastrous results. However, it was observed that dezincification often showed no marked tendency to occur preferentially adjacent to copper when relatively large areas of alpha/beta brass were exposed to the action of water, and galvanic corrosion of copper adjacent to silver brazing alloy was usually very localised (if observed at all). Hence the ratio of areas can only have been a contributory factor. The main reason for the rapid deterioration of alpha/beta brass is probably the marked difference in composition of the two phases and the small distances which separate the phases. So the galvanic couples alpha/beta and (subsequently) alpha/spongy copper can work over very small distances with great efficiency.
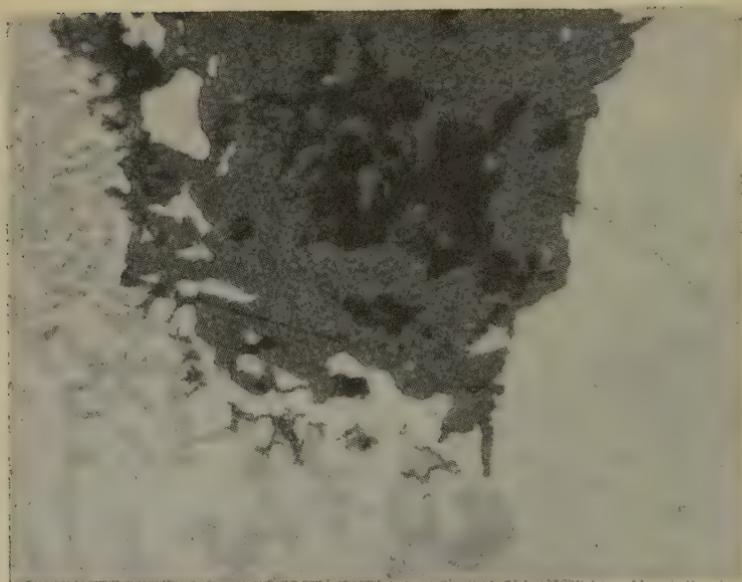
Fig. 7—A "Silbralloy"/copper junction from another part of the same cylinder shown in Fig. 6. Preferential attack on Cu$_3$P phase (darker). × 1,300. Unetched.

No failure due to corrosion of silver brazing alloy or interaction between silver brazing alloy and copper was seen. The most commonly encountered silver brazing alloys were "Silbralloy" and "Silfos" – both are copper-silver-phosphorus alloys. "Easyflo" appears to be rare – possibly because of cost, and also because flux is required.

All the evidence indicates that, in the absence of dezincification troubles, rapid hot-water cylinder corrosion is caused by under-deposit attack. The active deposit usually consists of corrosion product; even when some foreign body such as a mud particle initiates attack, corrosion product soon accumulates. Thus it seems probable that the chemical species normally present in water supplies affect hot-water cylinder corrosion chiefly by their influence on corrosion product deposit formation.

The effectiveness of raising pH and/or phosphate treatment as an anti-corrosive water treatment may depend primarily on the changed solubility of certain copper compounds and the consequent avoidance of corrosion products deposits. The data available from Invercargill was insufficient to determine whether the marked improvment was caused by phosphate or alkali or both. It is doubtful if under-deposit corrosion, once initiated, can be stopped by water treatment. Both the Lower Hutt failure and evidence gathered at Invercargill suggest that once localised corrosion has started it proceeds largely independent of the relatively small changes in external conditions which are brought about by water treatment.

English experience on the behaviour of alpha/beta brass joints appears to have been similar to that in New 'Zealand, and B.S. 699 specifically recommends copper-silver-phosphorus alloys, and bans brass alloys which are subject to dezincification.

CONCLUSION

Owing to their susceptibility to dezincification, alpha/beta brasses are not generally suitable for use in fabricating brazed joints in hot-water cylinders. It appears very likely that in some districts dezincification progresses so slowly that when a cylinder brazed with alpha/beta brass finally does fail its life is considered satisfactory. However, it is almost certain that in such districts cylinders brazed with copper-silver-phosphorus alloys would last considerably longer.

Copper-silver-phosphorus brazing alloys such as "Silbralloy" and "Silfos" are satisfactory.

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NEW ZEALAND

JOURNAL OF AGRICULTURAL RESEARCH

Volume 3, No. 5, October 1960

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NEW ZEALAND JOURNAL OF SCIENCE

Volume 4, No. 1, March 1961

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